

Influence of bar opening on the fish fauna of Toby Inlet

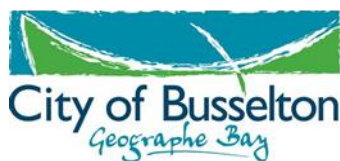
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Cover photographs. Aerial photograph of the mouth of Toby Inlet and the Deadwater.

Photographs by Tom Hoy.

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Executive summary

Despite its catchment being subjected to substantial anthropogenic modification to support agriculture and urban development, Toby Inlet is highly valued by the community and considered to provide important habitat for fish and waterbirds. This study aimed to quantify these perceived biological values by determining the fish and benthic macroinvertebrate species present in the system and elucidate whether the number of species, abundance, diversity and faunal composition differed among regions of the estuary and/or changed when the bar at the mouth of Toby Inlet was closed (November 2017) and open (March 2018).

A total of 12,438 fish from 17 species were recorded in the shallow, nearshore waters of Toby Inlet across the two sampling occasion. Most of these are short-lived, small-bodied (< 50 mm total length), belong to the atherinids and gobys and complete their life cycle within the estuary. While the mean number of species, density and diversity remained similar among regions and seasons, faunal composition changed following the opening of the bar, but this was mainly due to the timing of reproductive cycles of these typically short-lived (1 year life cycle) species. It also reflects the fact that these species are generally highly euryhaline and able to tolerate even the hypersaline conditions that occurred in the upper region in March.

In contrast to nearshore waters, the 620 fish caught and released in the offshore waters comprised mainly marine species. The composition of this fauna changed markedly following the opening of the bar as only four species were recorded in November, increasing to 12 after the bar had been open for several months. All of the species recorded only in March spawn in marine waters and where able to recruit to the estuary as the bar was open and salinities had increased markedly, facilitating their survival in the estuary. The changes in faunal composition were mirrored by changes in mean number of species and catch rates, both of which were greater in March. Regional differences in fauna were also detected, due to the declining

influence of these marine species in the upper estuary and Deadwater. This latter region contained an impoverished fauna due to its shallow depths and limited connectivity to the ocean (the Station Gully mouth was closed throughout the study) and the lower region of Toby Inlet.

The benthic macroinvertebrate fauna, which was only sampled in November, comprised a range of insect, crustacean, annelid and mollusc species typically found in either wetland or estuarine environments. This reflects the low salinities at the time of sampling (2-8) and the connectivity of the system to wetlands upstream of Caves Road Bridge. While some, annelids and molluscs were found in each of the four regions, some wetland insects and amphipods were more common and abundant in the upstream regions (upper estuary and the Deadwater), whereas the reverse was true for several estuarine species of annelids and a bivalve.

The opening of the bar, allowed the immigration and emigration of fish species from the estuary and increased salinity. This influenced the fish fauna in offshore more than nearshore waters, as most of these species breed in marine waters and thus require the bar to be open to be able to recruit to the estuary. As invertebrates were only sampled in November, when salinities, were very low, there would be value in repeating the sampling when salinities were greater to elucidate what effect bar opening may have on that fauna, particularly as many species of fish and birds use this fauna as a critical food source. It should be noted that sampling was only conducted in areas of downstream of Caves Road Bridge, but that an option to construct a second mouth in the upper region of Toby Inlet would influence the fauna in that region and thus there is a need to collect some baseline data from that area to allow the potential impacts to be explored. However, the data in this report provide a robust baseline against which future changes in the fauna of Toby Inlet could be detected and also may help in the development of management strategies to improve the health of this highly-valued estuary.

Introduction

Estuaries have traditionally been a focal point for human colonization and development, as they provide an abundant source of food, both directly from their waters (fisheries) and indirectly through agriculture on surrounding fertile land, sheltered harbours for ships and key transport route for the import and export of goods, all of which have resulted in the development of settlements and industry (Wilson, 1988; Lotze et al., 2006). Indeed, seven of 10 of the world's largest megacities are located on or close to estuaries, including Tokyo, Jakarta, Shanghai, Seoul and New York (Potter et al., 2015b). Anthropogenic influence on these systems include, in particular, land clearing, dredging, spoil dumping, canal cutting and reclamation, all of which can result in the loss or modification habitats in the subtidal, intertidal or surrounding catchment, and eutrophication and hypoxia, brought about by the input of large amounts of nutrients from surrounding agricultural land, urban development and sewage (Kennish, 2001; 2002; Bricker et al., 2008; Rabalais et al., 2010).

Estuaries provide a range of ecosystem services including direct-use values, *e.g.* raw materials and food, non-consumptive direct-use values, *e.g.* tourism, recreation, education and research, and indirect-use values, *e.g.* coastal protection, erosion control, water catchment and purification, maintenance of beneficial species and carbon sequestration (Barbier, 2007; Barbier et al., 2010). For example, estuaries are typically regarded as nursery areas for a range of fish and crustacean species (Beck et al., 2001; Sheaves et al., 2014) and those fish that use estuaries during some stage of their life cycle contribute >75% of commercial fish catch in Australia, and in some regions up to 90% of all recreational angling catch (Creighton et al., 2015). The value of the ecosystem services provided by estuaries was estimated to be \$US 28,916 ha/y⁻¹, which is five times greater than the corresponding value for tropical forests and only exceeded by tidal marsh/mangroves and coral reefs (Costanza et al., 2014).

While the ecological and biological values of estuaries impact on the socio-economic systems (*i.e.* human well-being) via ecosystems services, anthropogenic modification acts as driver of change on the system (Fisher et al., 2009; Maes et al., 2016). As a result of anthropogenic modifications temperate estuaries are regarded as the most degraded of all marine ecosystems (Jackson et al., 2001), with those in microtidal estuaries, where tidal range < 2 m (Tweedley et al., 2016b) being particularly susceptible to degradation (Tweedley et al., 2014b; 2015). Furthermore, Warwick et al. (2018) argue that microtidal estuaries in Mediterranean and arid climates are intrinsically less robust than well-flushed macrotidal estuaries, meaning that any increase in anthropogenic perturbation will further decrease the health of a system in which the biota already experiences ‘natural’ stress (Elliott and Quintino, 2007). Thus these estuaries warrant special management measures that recognise their critical vulnerability to pollution and climate change.

In south-western Australia, microtidal conditions and highly seasonal rainfall results in some estuaries become disconnected from the ocean either intermittently, seasonally, normally (*i.e.* several years at a time) and, in some cases, permanently, after which they are no longer regarded as estuaries (Tweedley et al., 2017b; Hoeksema et al., 2018). Moreover, sustained warming and drying trends have caused dramatic declines in freshwater flows of up to 70% since the 1970s, leading to warming and ‘marinisation’ of estuaries; extended closure of periodically-open systems; an increased frequency and severity of hypersaline conditions; enhanced water column stratification and hypoxia; and reduced flushing and greater retention of nutrients (Hallett et al., 2018). This is reflected in long-terms changes in the characteristics of their faunal assemblages (e.g. Wildsmith et al., 2009; 2011; Veale et al., 2014; Potter et al., 2016).

Toby Inlet as a small, ~6 km long and 200 m wide, and very shallow (< 1 m deep) estuary located near the town of Dunsborough in south-western Australia. The hydrological characteristics of this system have been modified radically as a result of over 200 years of land-clearing, river diversion and drainage modification, resulting in the system receiving far less fluvial discharge (Frazer and Hall, 2018). The estuary today is split into two main components Toby Inlet, which is fed by a 33 km² catchment via the Dunsborough Lakes and a range of streams, and the Deadwater, which receives freshwater input from a straighten drain (Station Gully) created in the 1920s to improve drainage of agricultural land and drains an area of 110 km². Rainfall, ~ 800 mm per annum, is highly seasonal occurring in winter and spring and produced, on average, 12 and 58 GL y⁻¹ of streamflow into Toby Inlet and Station Gully, respectively, between 2010 and 2015 (Frazer and Hall, 2018). Both Toby Inlet and Station Gully have separate openings to the ocean, which are reported to open seasonally and close due to declining freshwater flow and the longshore transport of sand (Brearley, 2005). Historically, the mouth of Toby Inlet was further east and the Deadwater was incorporated into Toby Inlet, but today the two water bodies are only connected by a small culvert.

While the estuary is highly valued by the local community, the reduced flow, periodic connection with the ocean and shallow water depth, combined with the substantial input of nutrients from the surrounding agricultural and urban development (Department of Water, 2010), result the systems suffering from poor water quality. This has led to regular blooms of macroalgae and hypoxia during summer (Frazer and Hall, 2018) and acid sulphate soils, including monosulfidic black ooze (Ward et al., 2010). Multiple fish kills are reported to have occurred, with documented reports in 1983 and 2014 (Brearley, 2005; Frazer and Hall, 2018), although this is likely an underestimation.

Following an independent review of the current and future management of water assets in the Geographe catchment (Hart, 2014), the Revitalising Geographe Waterways project was established. This initiative aimed to improve the water quality, waterway health and management of Geographe Waterways (including the Vasse-Wonnerup Estuary, Lower Vasse River and Toby Inlet). In particular, a hydrological model and management plan for Toby Inlet were to be developed. The model predicted that (i) artificially maintaining the Toby Inlet mouth open resulted in 72% of the inlet being regularly tidally flushed, considerably more than maintaining the Station Gully mouth (36%). (ii) Artificially opening the Toby Inlet mouth to a sill elevation of less than -0.15 m AHD may cause the inlet to drain on low tides, leaving individual pools that may become warm and hypoxic and (iii) even with an open mouth, poor circulation (high residence time) remains in the upper parts of Toby Inlet, which, to ameliorate, would require another mouth to be constructed (Frazer and Hall, 2018).

Estuaries are dynamic environments and changes to the physico-chemical conditions can occur naturally, such as from seasonal differences in weather and climate and as a result of anthropogenic modification, *e.g.* bar breaching and dredging (Chuwen et al., 2009b; Hoeksema et al., 2018; Tweedley et al., 2018b). As characteristics of the fish and benthic macroinvertebrate fauna of estuaries have been shown to change in response to differing environmental variables (Ysebaert et al., 2003; França et al., 2009; Valesini et al., 2009), and that no baseline faunal data had been collected for Toby Inlet, the study had the following aims. (i) provide a quantitative description of the fish and benthic macroinvertebrate fauna of Toby Inlet, (ii) determine whether the number of species, abundance, diversity and faunal composition of the fish fauna of the shallow and deeper waters differed among regions of the estuary and when the bar of Toby Inlet was closed and open and (iii) if those of the invertebrate fauna differed between regions.

Materials and methods

Sampling regime

Fish faunas in the shallow, nearshore waters (< 1 m deep) of four regions of Toby Inlet were sampled during November 2017, when the bar at the estuary mouth was closed to the ocean, and in March 2018, when the bar was open, having been artificially breached on 11 December 2017. The four regions constituted the Lower, Middle and Upper Toby Inlet and the Deadwater (Figs 1, 2). Note that the bar at the mouth of Station Gully was closed for the duration of the study, having closed shortly before the fieldwork was undertaken. On each sampling occasion, two replicate seine net samples were collected at the six sites representing each of the three regions in Toby Inlet, while four sites were sampled in the Deadwater, due to this waterbody being smaller in size (Fig. 1). The seine net employed was 21.5 m long and consisted of two 10 m long wings (6 m of 9 mm mesh and 4 m of 3 mm mesh) and a 1.5 m long bunt made of 3 mm mesh and swept an area of ~ 116 m² (Fig. 3). Upon capture, all fish were immediately euthanized in an ice slurry, frozen and transported to the laboratory (Murdoch University Animal Ethics Permit R2987/17 and Department of Primary Industries and Regional Development exemption 2987). The total number of individuals of each fish species in each sample was then recorded and the total length of each individual measured to the nearest 1 mm, except when a large number of any one species was caught, in which case the lengths of a representative subsample of 50 fish were measured. Catches were converted to a density (individuals 100 m⁻²).

Fish in the adjacent offshore waters (~ 1 m deep) were sampled at four sites in each region, on both sampling occasions, using a sunken composite multifilament gill net (Fig. 3). The net, which has a total length of 160 m, comprised eight 20 m long panels, each with a height of 2 m and containing a different stretched mesh size, *i.e.* 35, 51, 63, 76, 89, 102, 115 or 127 mm. Gill nets were set for one hour, to minimise any fish deaths and allow the release of fish alive to the

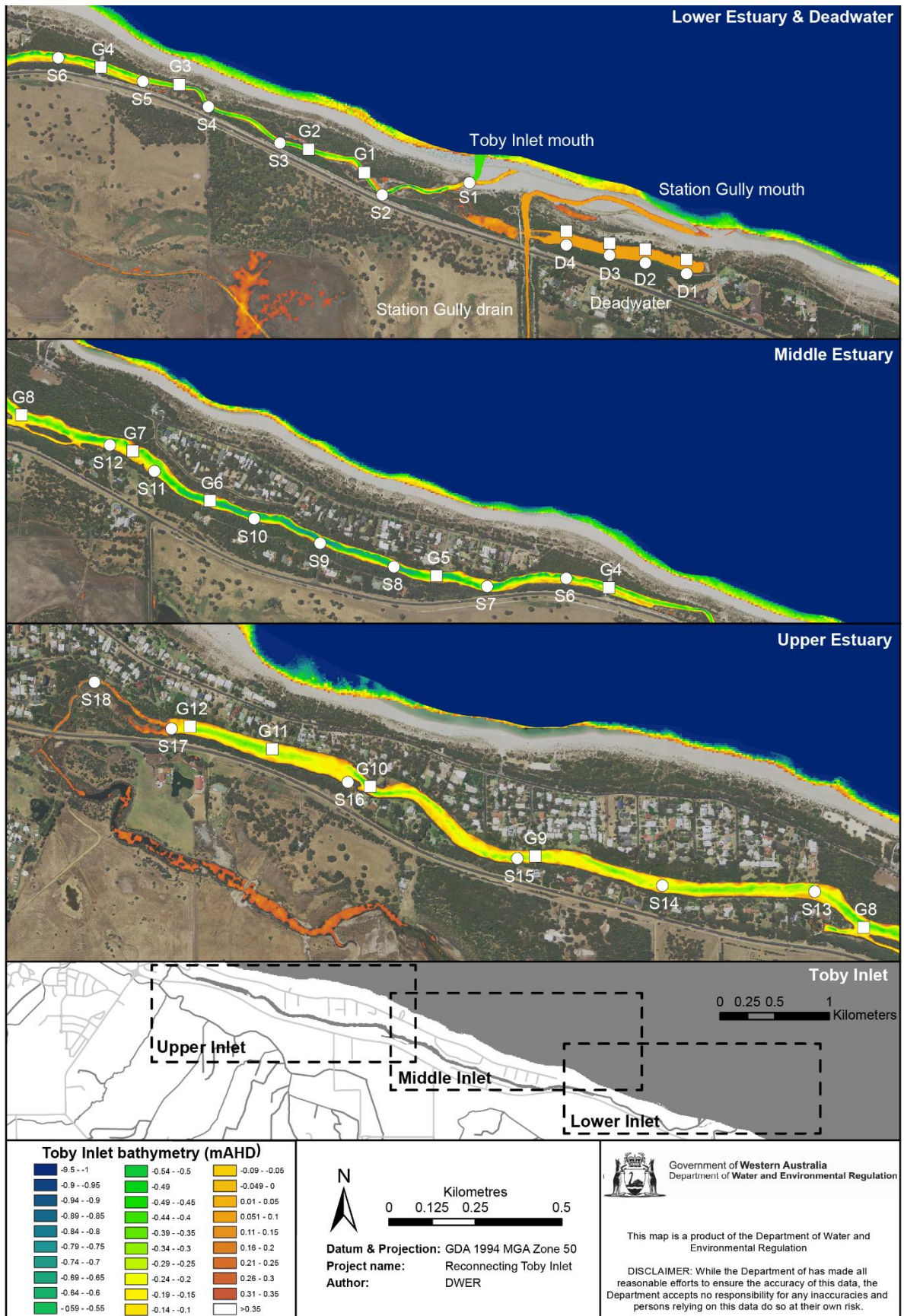


Fig. 1. Map of Toby Inlet showing the four regions of the estuary, the two mouths and the 16 nearshore sites (●) and 12 offshore sites (■) where sampling was conducted. Coloured scale denotes the bathymetry. Base map supplied by the Department of Water and Environmental Regulation.



Fig. 2. Photographs of Toby Inlet showing the (a) lower, (b) middle and (c) upper estuary and the (d) Deadwater in March 2018. Photographs taken by Tom Hoy.

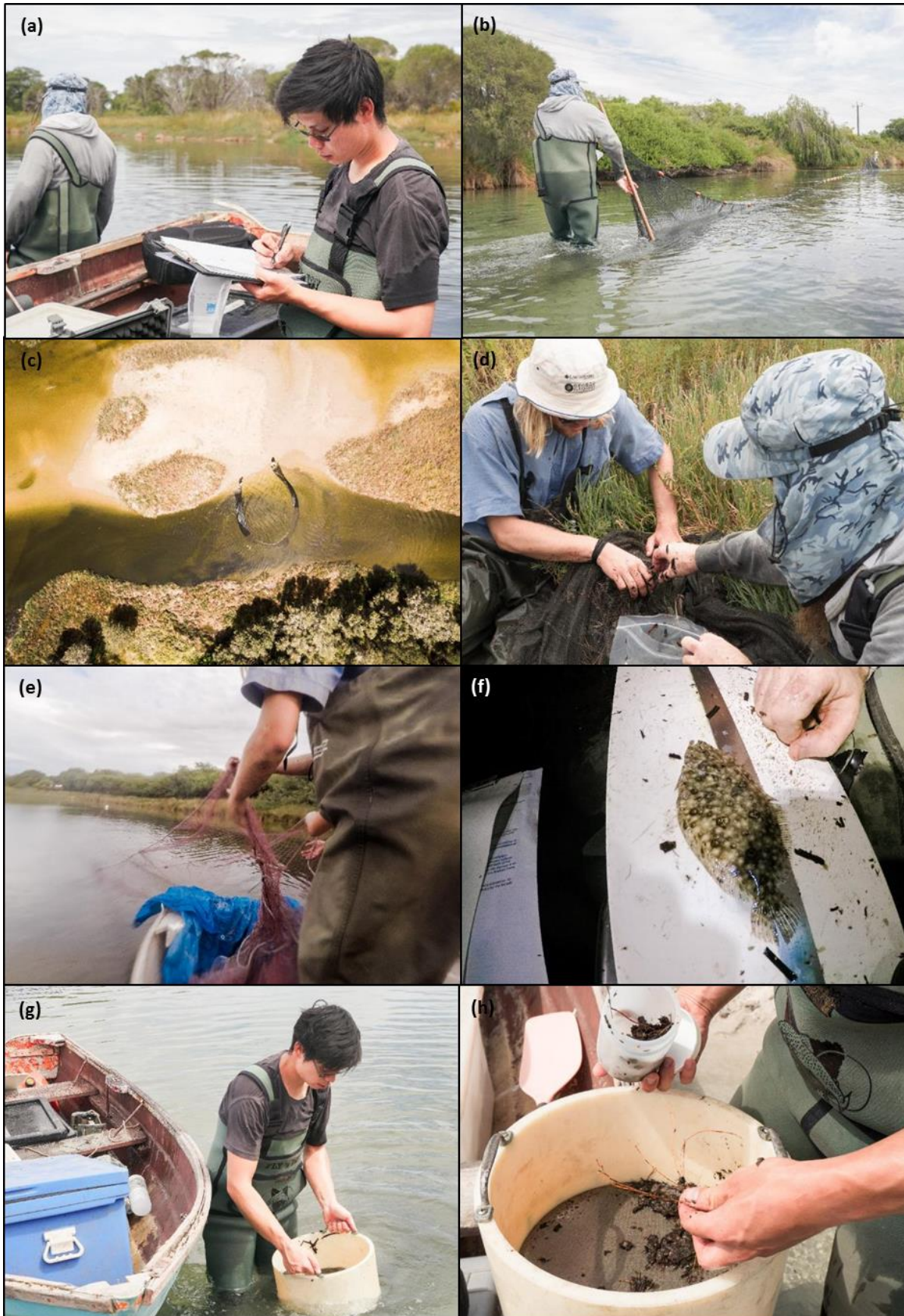


Fig. 3. Photographs of the fieldwork showing the (a) measurement and recording of water quality, (b-d) seine net sampling, (e, f) gill net sampling and (g, h) collection and sieving of benthic macroinvertebrates. Photographs taken by Tom Hoy.

water (Grixti *et al.*, 2010). Fish were identified to species, counted and measured to the nearest 1 mm before being released alive to the water. Abundances were recorded as catch rate h^{-1} .

Each fish species was also assigned to a life-cycle guild within a category according to the way in which it uses estuaries (Potter *et al.*, 2015a; 2015b). Definitions of the three categories and seven guilds relevant to the current study are as follows. **Marine category**, *i.e.* species that spawn at sea. *Marine straggler* (MS) guild, *i.e.* species that spawn at sea and typically enter estuaries sporadically and in low numbers and are most common in the lower reaches, where salinities typically do not decline far below ~35. *Marine estuarine-opportunist* (MEO) guild, *i.e.* species that spawn at sea and regularly enter estuaries in substantial numbers, particularly as juveniles, but can also use coastal marine waters (*e.g.* Geographe Bay) as alternative nursery areas. **Estuarine category**, *i.e.* species with populations in which the individuals complete their life cycles within the estuary. *Solely estuarine* (E) guild, *i.e.* species typically found only in estuaries. *Estuarine & marine* (E&M) guild, *i.e.* species represented by populations whose individuals complete their life cycle either in estuaries or coastal marine waters. *Estuarine & freshwater* (E&F) guild, *i.e.* species represented by populations whose individuals complete their life cycle either in estuaries or fresh water. **Freshwater category**, *i.e.* species that spawn in freshwater. *Freshwater straggler* (FS) guild, *i.e.* species that are found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries. *Freshwater estuarine-opportunist* (FEO), *i.e.* species found regularly and in moderate numbers in estuaries and whose distribution can extend well beyond the oligohaline sections of these systems. No species belonging to the **diadromous category**, *i.e.* species that migrate between the sea and freshwater, such as the Pouched Lamprey (*Geotria australis*) were recorded in this study.

Benthic macroinvertebrates were sampled in only November 2017 using an Ekman grab (Wildco, Yulee, Florida, USA) that collected substrata from an area of 225 cm² and sampled to a depth of 15 cm. Each sediment sample from each site was wet-sieved in the field and preserved in a 5% formalin mixture buffered in estuary water and, after at least one week, subsequently wet-sieved through a 500 µm mesh and stored in 70% ethanol. Using a dissecting microscope, any invertebrates found in a sample were removed from any sediment retained on the mesh and identified to the lowest possible taxonomic level. Catches were converted to a density (individuals 100 cm²).

Water temperature (°C), salinity and dissolved oxygen concentration (mg L⁻¹) were measured in the middle of the water column at each nearshore site at the time of sampling using a Yellow Springs Instrument 556 water quality meter (Fig. 3). Long-term salinity data from one site in each region of Toby Inlet was measured in January-April/May and October/November and December from January 2015 to May 2018 by the Department of Water and Environmental Regulation and extracted from the Water Information Reporting online database (<http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx>).

Statistical analyses

All statistical analyses were performed using the PRIMER v7 multivariate statistics software package (Clarke and Gorley, 2015) with the PERMANOVA+ add-on (Anderson et al., 2008).

Univariate analyses

A data matrix containing the single value for water temperature, salinity and dissolved oxygen concentration value recorded at each nearshore site on each of the two sampling occasions (November and March), was used to construct a separate Euclidean distance matrix for each water quality variable. Each matrix was subjected to two-way Permutational Multivariate

Analysis of Variance (PERMANOVA; Anderson et al., 2008) to determine whether that variable differed among Season (2 levels; November 2017 [bar closed] and March 2018 [bar open]) and Region (4 levels; Lower, Middle and Upper estuary and the Deadwater). In this and all other tests the null hypothesis of no significant difference among *a priori* groups was rejected if the test statistic (P) was < 0.05 . The percentage contribution made by the mean square for each main effect and interaction term to the total value for the mean squares was calculated to provide an estimate of the relative importance of each term in the model. When a significant difference was detected, a pairwise PERMANOVA test, together with a plot of the means and associated 95% confidence limits, was used to identify the pairwise combination of *a priori* groups responsible for that difference. Prior to undertaking these analyses, ‘draftsman plots’ of the values for each pair of water quality variables at each site in each season were examined visually to assess whether the values for each variable were heavily skewed and, if so, which type of transformation would ameliorate that effect. These plots demonstrated that none of the variables required any transformation.

Separate data matrices were also constructed from the density of fish (100 m^{-2}) and benthic invertebrates (100 cm^{-2}) from nearshore waters and catch rate (h^{-1}) of fish from offshore waters. Note that, in the case of the nearshore fish fauna, data from the two samples collected from each site on each sampling occasion were averaged prior to analysis. Each of the three matrices was, in turn, subjected to the DIVERSE routine to calculate the number of species, total density/catch rate and Simpson’s Diversity Index (Somerfield et al., 2008). As above, the values of the number of species, total density and Simpson’s Diversity Index for each of the three faunal assemblages was subjected to ‘draftsman plots’, which indicated that the total density of fish in nearshore waters required a $\log_e(x+1)$ transformation, while the catch rate of fish in offshore waters and density of benthic invertebrates needed to be square-root transformed. Each univariate measures of diversity from each faunal assemblage was then used

to construct a Euclidean distance matrix and subjected to the PERMANOVA design described above, except for the benthic macroinvertebrates where a one-way PERMANOVA was employed as data were only collected in November 2017.

Multivariate analyses

Prior to constructing a Bray-Curtis resemblance matrix, the variability in the average numbers of individual fish species present in each site in the nearshore waters of Toby Inlet within each season and region combination was used to carry out dispersion-weighting for each species, which down-weights the effects of those species whose numbers exhibited large differences among replicate samples (Clarke et al., 2006). This was achieved by dividing the counts for each species by its mean index of dispersion (*i.e.* average of the variance to mean ratio in replicate samples within a region) and ensures that all species have similar variability structures, and prevents the analyses becoming dominated by large outliers. These data were then square-root transformed to down-weight the contributions of species with consistently high values in relation to those with consistently low values and averaged to provide a single value for each species at each site. This pre-treatment combination has proven effective when analysing faunal communities in estuaries, where the prevalence of juvenile and schooling fish species are typically high (Clarke et al., 2014b; Veale et al., 2014). The catches rates of each fish species in the offshore waters were less variable than those in the corresponding shallower waters and thus only subjected to a square-root transformation before being used to construct a Bray-Curtis resemblance matrix. While the benthic macroinvertebrate species from nearshore waters were subjected to dispersion-weighting and a square-root transformation, the former pre-treatment was conducted using only the variability among regions as this fauna was only sampled in November 2017.

The Bray-Curtis resemblance matrices for the fauna data were subjected to the same two-way (nearshore and offshore fish) and one-way (benthic macroinvertebrates) PERMANOVA designs described above. When a significant difference in any main effect or interaction term was detected, a pairwise PERMANOVA was conducted to identify the particular pairwise comparison(s) that were responsible for the difference.

To visualize the patterns exhibited each of the three faunas non-metric MultiDimensional Scaling (nMDS) ordination plot (Clarke, 1993) was constructed from the above Bray-Curtis resemblance matrices. In the case of the nearshore fish fauna, where a Season \times Region was detected, a centroid nMDS ordination plot was produced using a distances among centroids matrix, which creates averages in the 'Bray-Curtis space' from points for the four or six sites representing region in each season (Lek et al., 2011).

When a significant difference between the faunal compositions of *a priori* group(s) was detected, Similarity Percentages (SIMPER; Clarke et al., 2014a) were used to identify those species that typified the species composition of each group and those that were responsible for distinguishing between the fish compositions in each pair of groups. Shade plots, derived from the pre-treated and averaged data, were constructed and used to visualise the trends exhibited by the abundance of the fish and benthic macroinvertebrate species. Note the configuration of the plot was based on the significance of the main effect and/or interactions determined by the above PERMANOVA tests. Each shade plot is a simple visualisation of the frequency matrix, where a white space for a species demonstrates that a particular species was not collected, while the depth of shading from grey to black is linearly proportional to the abundance of that species (Clarke et al., 2014b; Valesini et al., 2014). Species (*y* axis) and Seasons, Regions or Season \times Region combinations (*x* axis) were clustered based on their Bray-Curtis similarities and placed in optimum serial order (Clarke et al., 2014a).

Results

Water quality

Among the ‘summer’ months in 2015, 2016, 2017 and 2018 the salinity in all regions of Toby Inlet was typically > 30 (Fig. 4). Notable exceptions, however, occurred in November 2016 when salinity reached as low as 8 and 10 in the upper and middle estuary, respectively, and, during the current study (*i.e.* October to December 2017), when salinities in all regions declined markedly from <36 in October to as low as ~ 1 . Hypersalinity (*i.e.* > 40) was recorded in all regions except the lower estuary, but occurred predominantly in the upper estuary. Salinities in this region exceeded 40 on 11 of the 26 sampling occasions and reached as high as 48 (Fig. 4).

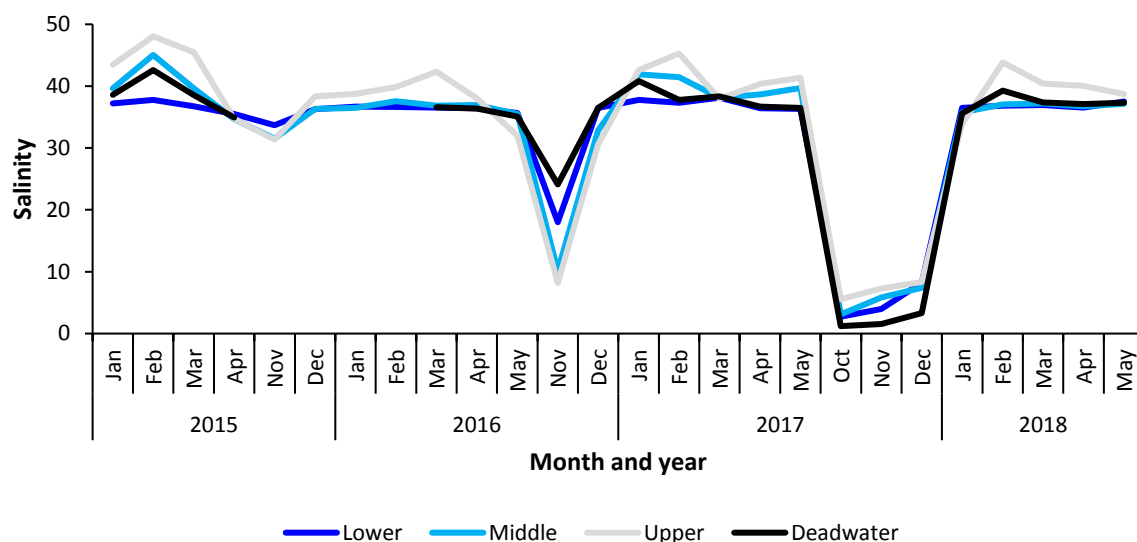


Fig. 4. Mean salinity recorded bi-weekly in the ‘summer’ months (October/November – April/May) at a site in each region of Toby Inlet between January 2015 and May 2018. Data supplied by the Department of Water and Environmental Regulation.

Water temperature was shown by two-way PERMANOVA to differ significantly among Season, Region and the Season \times Region interaction (all $P = 0.001$), with each term contributing a similar portion to the total variability (%MS 30-35; Table 1a). Temperature values ranged between 23.4 and 28.5 °C, with those from the lower and middle estuary staying relatively consistent between seasons at ~ 25 , whereas those in the Deadwater and particularly

Table 1. Mean squares (MS), percentage contribution of mean squares to the total mean squares (%MS), pseudo-F ratios (pF) and significance levels (*P*) from two-way and one-way PERMANOVA tests on the (a) water quality (*i.e.* water temperature, salinity and dissolved oxygen concentration) and univariate measures of diversity (*i.e.* number of species, total density/catch rate and Simpson's diversity index) of the (b) nearshore fish fauna, (c) offshore fish fauna and (d) benthic invertebrate fauna from the four regions of Toby Inlet in November 2017 and/or March 2018. *df* = degrees of freedom. Significant results are highlighted in bold and those with a %MS ≥ 10 shaded in grey.

(a) Water quality		Water temperature				Salinity				Dissolved oxygen conc.			
Source	df	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P
Season	1	18.62	35.68	25.5	0.001	13,604.00	97.53	18,165	0.001	89.27	94.05	206.0	0.001
Region	3	16.87	32.32	23.1	0.001	213.39	1.53	285	0.001	4.98	5.24	11.5	0.001
Season \times Region	3	15.97	30.60	21.9	0.001	130.27	0.93	174	0.001	0.24	0.25	0.6	0.663
Residual	40	0.73	1.40			0.75				0.43	0.25		

(b) Nearshore fish		Number of species				Total density (100 m2)				Simpson's diversity				Faunal composition			
Source	df	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P
Season	1	0.08	2.62	0.04	0.848	0.65	13.13	0.61	0.439	0.02	19.84	0.73	0.404	7,077	36.68	4.69	0.001
Region	3	0.72	22.69	0.36	0.783	0.57	11.40	0.53	0.652	0.02	14.95	0.55	0.657	5,898	30.57	3.91	0.001
Season \times Region	3	0.36	11.34	0.18	0.900	2.69	53.87	2.49	0.080	0.04	38.08	1.40	0.236	4,810	24.93	3.19	0.001
Residual	40	2.02	63.35			1.08	21.60			0.03	27.13			1,509	7.82		

(c) Offshore fish		Number of species				Total catch rate (1 h)				Simpson's diversity				Faunal composition			
Source	df	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P
Season	1	24.50	55.55	8.22	0.008	78.13	5.90	0.41	0.523	0.08	31.63	1.75	0.192	4,578	29.39	2.87	0.016
Region	3	14.54	32.97	4.88	0.006	998.75	75.43	5.26	0.015	0.11	43.40	2.39	0.081	6,694	42.98	4.20	0.001
Season \times Region	3	2.08	4.72	0.70	0.559	57.38	4.33	0.30	0.818	0.02	6.84	0.38	0.777	2,708	17.39	1.70	0.092
Residual	24	2.98	6.75			189.79	14.33			0.05	18.12			1,595	10.24		

(d) Invertebrates		Number of species				Total density (100 cm2)				Simpson's diversity				Faunal composition			
Source	df	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P	MS	%MS	pF	P
Region	3	5.15	32.74	0.49	0.715	19.01	52.75	1.12	0.395	0.06	57.76	1.37	0.278	4,846	71.13	2.46	0.001
Residual	18	10.58	67.26			17.03	47.25			0.04	42.24			1,967	28.87		

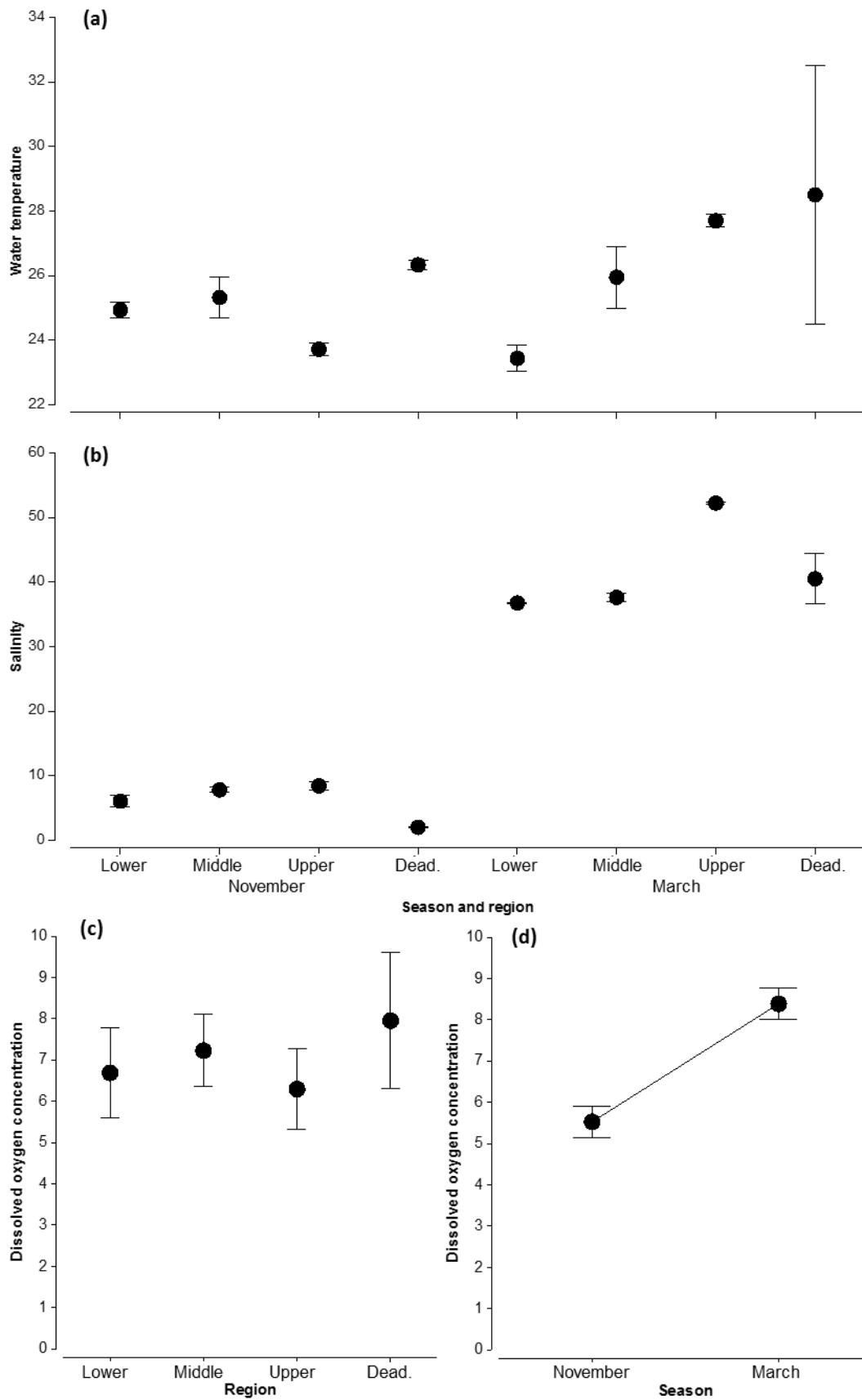


Fig. 5. Mean (a) water temperature (°C) and (b) salinity in each region of Toby Inlet in each season and mean dissolved oxygen concentration (mg L⁻¹) in each (c) region and (d) season. Error bars represent \pm 95% confidence limits.

the upper estuary increased (*i.e.* 26.3 to 28.5 and 23.7 to 27.7, respectively; Fig. 5a).

While salinity differed among both main effects and their interaction (all $P = 0.001$), Season was the most influential factor, representing 97.5% of the total mean squares (Table 1a). Salinities were far greater in March ranging from 37 to 52, than in November where they were all \leq eight (Fig. 5b). The significant regional difference was due to salinities increasing in an upstream direction (*i.e.* a reverse salinity gradient) in both seasons, with this trend being more marked in March. Two-way PERMANOVA demonstrated that dissolved oxygen concentration differed between both Season and Region, with the former main effect being more influential (Table 1a). Oxygen concentrations were always well above hypoxia (*i.e.* 2 mg L⁻¹) and greater in the Deadwater than the other regions, which were all fairly similar (~8 vs ~7 mg L⁻¹, respectively; Fig. 5c). Values for this water quality variable were also substantially higher in March, when the bar was open, than in November (Fig. 5d).

Nearshore fish fauna

Contributions of fish species

A total of 12,438 fish from 17 species representing nine families were collected from the 84 samples from the nearshore waters of Toby Inlet (Table 2). Among the families, the Atherinidae and Gobiidae were the most speciose, comprising five and three species, respectively. Nine of the 17 species recorded are able to complete their lifecycle in estuaries (*i.e.* estuarine & freshwater, solely estuarine and estuarine & marine species), with marine species (*i.e.* marine estuarine-opportunists) and freshwater species (*i.e.* freshwater estuarine-opportunists), comprising the remaining six and two species, respectively (Table 1).

Eleven species were recorded in November, with the atherinids *Leptatherina wallacei* and *Atherinosoma elongata* being the most abundant, representing 86 and 79% in November 2017

Table 2. Mean number of individuals (100 m⁻²; X), standard error (SE), percentage contributions to the total catch (%C), ranking by contribution (R), mean total length (L, mm) and length range (Lr) of each fish species caught in the nearshore waters of Toby Inlet in November 2017 (bar closed) and March 2018 (bar open). Those species that contributed ≥ 5% of the total catch in each month are shaded in grey. The estuarine usage functional group (EUFG) to which each species belong is provided (FEO, freshwater estuarine-opportunist; EF, estuarine & freshwater; E, solely estuarine; EM, estuarine & marine; MEO, marine estuarine-opportunist) along with the total number of species, total density (fish 100 m⁻²), number of individual fish recorded and the number of samples collected. Species ranked by total abundance across the two seasons.

Species	EUFG	November						March					
		X	SE	%C	R	L	Lr	X	SE	%C	R	L	Lr
<i>Leptatherina wallacei</i>	EF	74.82	73.54	73.12	1	40	12-69	106.69	67.14	69.74	1	33	20-63
<i>Atherinosoma elongata</i>	E	13.46	3.13	13.16	2	41	19-66	13.49	6.72	8.82	2	36	19-62
<i>Pseudogobius olorum</i>	EF	3.47	1.62	3.39	3	32	19-56	9.89	5.60	6.47	4	27	14-47
<i>Gambusia holbrooki</i>	FEO	2.36	0.99	2.31	6	26	15-47	10.16	5.53	6.64	3	24	16-36
<i>Afurcagobius suppositus</i>	EF	3.24	1.19	3.17	4	31	15-66	5.87	3.58	3.84	5	32	16-51
<i>Favonigobius lateralis</i>	EM	3.10	1.62	3.03	5	43	17-65	2.81	1.43	1.84	6	30	14-51
<i>Leptatherina presbyteroides</i>	EM							2.63	2.30	1.72	7	32	15-51
<i>Amniataba caudavittata</i>	E	1.09	0.74	1.06	7	82	82-82	0.18	0.15	0.12	10	95	30-154
<i>Pelates octolineatus</i>	MEO							0.51	0.47	0.34	8	29	18-37
<i>Mugil cephalus</i>	MEO	0.31	0.50	0.30	8	-	-	0.18	0.27	0.12	10	105	95-113
<i>Rhabdosargus sarba</i>	MEO	0.18	0.21	0.18	10	29	27-30	0.06	0.07	0.04	14	38	38-38
<i>Galaxias occidentalis</i>	FEO	0.25	0.22	0.24	9	49	40-52						
<i>Craterocephalus mugiloides</i>	E							0.23	0.19	0.15	9	40	34-47
<i>Sillago berrus</i>	MEO							0.12	0.14	0.08	12	22	19-28
<i>Pseudorhombus jenynsii</i>	MEO							0.10	0.11	0.07	13	-	-
<i>Atherinomorus vaigiensis</i>	MEO							0.04	0.07	0.03	15	43	38-48
<i>Acanthopagrus butcheri</i>	E	0.04	0.05	0.04	11	124	116-132						
Number of species								11					
Total density								102.32					
Total number of fish								4,985					
Number of samples								42					

and March 2018, respectively. Other notable contributions were made by the gobiid *Pseudogobius olorum* and the introduced poeciliid *Gambusia holbrooki*, individuals of which contributed ~6% to the total number of fish caught in March 2018 (Table 2). The total density of fish increased by ~50% between November and March, as a result of the densities of *L. wallacei*, *P. olorum*, *G. holbrooki* and another gobiid, *Afurcagobius suppositus*, all increasing markedly (42-330%).

Number of species, total density and diversity

Neither the mean number of species, total density or Simpson's index were shown by PERMANOVA to differ significantly among either Season, Region or the interaction between these main effects (Table 1b). The mean number of fish species in the nearshore waters ranged only between ~5 in the Deadwater and upper estuary to ~6 in the lower and middle estuary, and there was an even smaller difference between each season for each region (*i.e.* 0-0.5). Total densities varied markedly among Season and Region ranging from 53-356 fish 100 m⁻², however, while the mean values of 264, 297 and 356 were recorded in the lower estuary in November and upper estuary and Deadwater, respectively, in March, these values were associated with a large amount of variability (*i.e.* 95% confidence limits = 0-700 fish 100 m⁻²). Thus, the extents of these limits overlapped considerably with the means density in all other season and region combinations (*i.e.* 53-92 fish 100 m⁻²). Values for Simpson's index ranged only from 0.45 to 0.65 and exhibited no conspicuous trend among seasons or regions.

Faunal composition

A significant difference in fish faunal composition for each term in the model was detected by PERMANOVA ($P = 0.001$; Table 1b). Among these, Season contributed the largest component of the mean squares (~37%), followed by Region and the Season \times Region interaction (31 and

25%, respectively). Due to the presence of an influential interaction term, pairwise PERMANOVAs were conducted to compare faunal composition among regions in each season.

In November 2017, when the bars of both Toby Inlet and Station Gully were closed, the nearshore fish fauna of the Deadwater differed significantly from that of all other regions as did those of the lower vs upper estuary (Table 3). The former difference is shown on the centroid nMDS ordination, where it lies at the bottom on the plot, well separated from the points representing the other three regions (Fig. 6). In this season the Deadwater contained two species not recorded in any other region, *i.e.* the sparid *Rhabdosargus sarba* and galaxiid *Galaxias occidentalis*, as well as greater densities of the gobiids *A. suppositus* and *Favonigobius lateralis* (Fig. 7). In contrast, the three regions of Toby Inlet all contained larger and more consistent catches of *A. elongata*, *G. holbrooki*, *P. olorum* and the terapontid *Amniataba caudavittata*. The less pronounced differences between the lower and upper estuary were due to greater densities of *F. lateralis* and *A. caudavittata* in the former region and of *A. elongata* and *P. olorum* in the latter (Fig. 7).

Table 3. *t* statistic and significance level (*P*) values derived from a pairwise PERMANOVA test on the Season × Region interaction of the fish faunal composition of the nearshore waters of Toby Inlet. Significant pairwise comparisons (*P* < 0.05) are highlighted in grey.

(a) November	Lower	Middle	Upper	(b) March	Lower	Middle	Upper
Middle	1.378			Middle	1.604		
Upper	1.655	1.287		Upper	2.603	1.962	
Deadwater	1.871	2.013	2.161	Deadwater	2.208	1.431	1.54

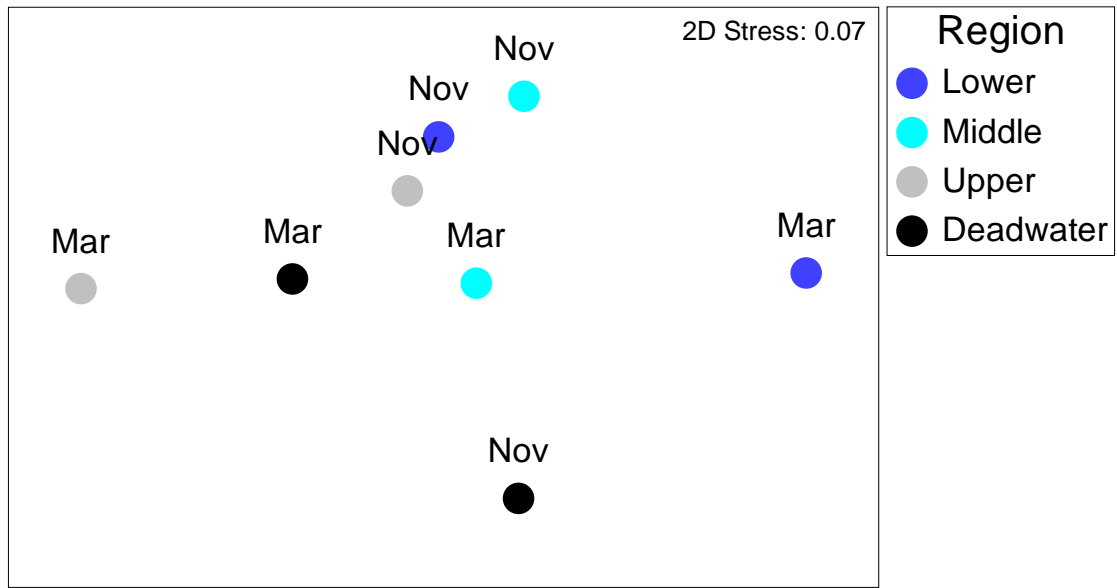


Fig. 6. Centroid non-metric Multidimensional Scaling ordination plot, derived from distance among centroid matrices constructed from a Bray-Curtis resemblance matrix of the dispersion-weighted and square-root transformed density of each fish species in the nearshore waters of the four regions of Toby Inlet in November 2017 (bar closed) and March 2018 (bar open).

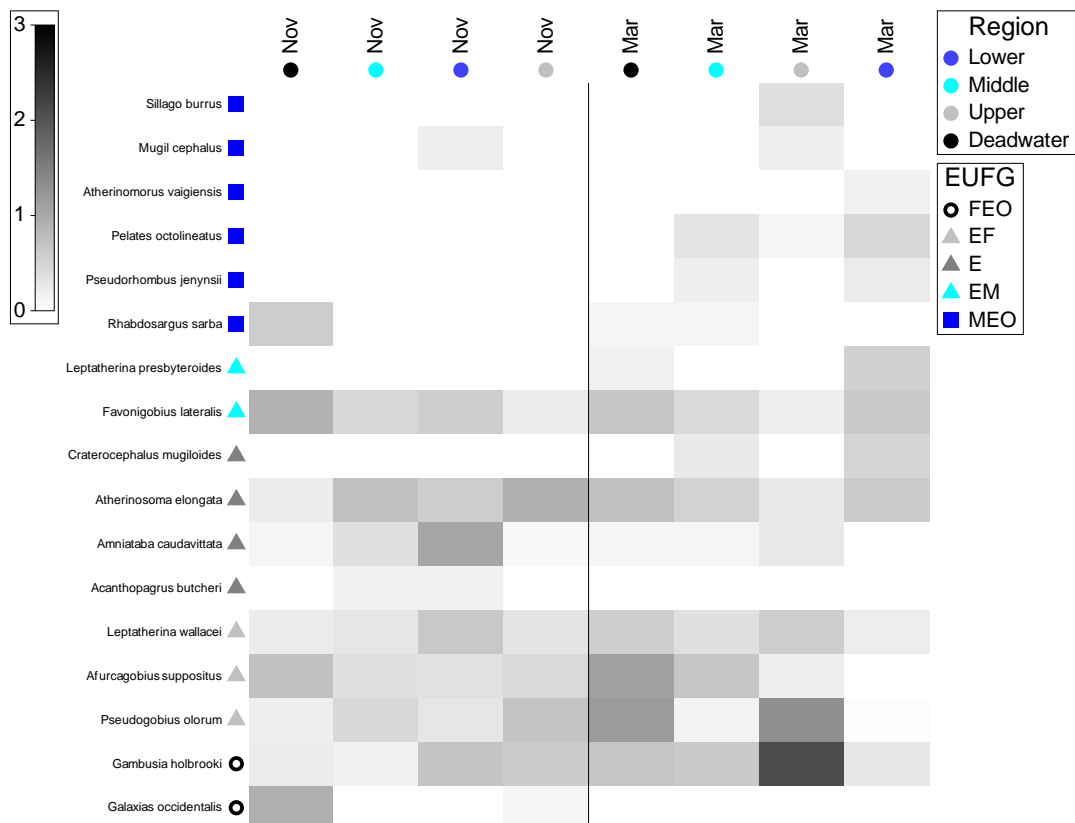


Fig. 7. Shade plot of the dispersion-weighted and square-root transformed average densities (fish 100 m⁻²) data for each species recorded in the nearshore waters of each of the four regions of Toby Inlet in November 2017 (bar closed) and March 2018 (bar open). White space indicates that a species was absent, and the shades of grey are proportional to the transformed abundances. The estuarine usage functional group (EUFG) to which each species belongs is also provided; see Table 2 for the full guilds.

Offshore fish fauna

Contributions of fish species

Gill netting in the offshore waters of Toby Inlet yielded 620 individuals, belonging to 12 species representing nine families (Table 4). The Mugilidae, Sparidae and Terapontidae were the only families to be comprise more than one species. In contrast to the nearshore waters, only two of the species recorded are able to spawn in estuaries, with nine of the remaining 10 species being marine estuarine-opportunists and the other a marine straggler. The total number of species recorded increased from only four when the bar of Toby Inlet was closed in November to 12 in March, following the artificial breaching of the bar at the mouth of the estuary. While the two estuarine species, namely *A. caudavittata* and the sparid *Acanthopagrus butcheri* were present in both seasons, the additional eight species recorded in March were all from marine EUFGs (Table 4). The above two species together with the mugilids *Mugil cephalus* and *Aldrichetta forsteri* comprised the entirety of the offshore fish fauna in November and almost 70% in March, with the terapontid *Pelates octolineatus* comprising > 20% to the total number of individuals in the latter season.

Number of species, total catch rate and diversity

PERMANOVA detected a significant difference between the mean number of species with Season and Region, with the former main effect making a larger contribution to the percentage mean squares (*i.e.* 46 vs 33%; Table 3c). A greater mean number of species was recorded in March than in November (4.3 vs 2.6; Fig. 8a) and, among regions, the value of 1.6 for the Deadwater was significantly less than those for the upper and middle estuary (~3.6) and lower estuary (4.8; Fig. 8b). Mean catch rate differed only among Region ($P = 0.015$; Table 3c), with, once again, the value for the Deadwater (2.8 fish h⁻¹) being substantially less than those in the three regions of Toby Inlet, *i.e.* 23.0-26.4 fish h⁻¹; Fig 8c). Simpson's diversity did not differ significantly with any term with most values ranging between 0.4 and 0.6 (data not shown).

Table 4. Mean catch rate (h^{-1} ; X), standard error (SE), percentage contributions to the total catch (%C), ranking by contribution (R), mean total length (L, mm) and length range (Lr) of each fish species caught in the offshore waters of Toby Inlet in November 2017 (bar closed) and March 2018 (bar open). Those species that contributed $\geq 5\%$ of the total catch in each month are shaded in grey. The estuarine usage functional group (EUFG) to which each species belong is provided (E, solely estuarine; MEO, marine estuarine-opportunist; MS, marine straggler) along with the total number of species, total catch rate (h^{-1}), number of individual fish recorded and the number of samples collected. Species ranked by total abundance across the two seasons.

Species	EUFG	November						March					
		X	SE	%C	R	L	Lr	X	SE	%C	R	L	Lr
<i>Mugil cephalus</i>	MEO	6.5	1.7	36.5	1.0	177	100-445	4.4	2.1	20.9	2.5	194	112-410
<i>Amniataba caudavittata</i>	E	5.7	2.2	31.9	2.0	175	95-243	3.9	1.2	18.5	4.0	186	115-242
<i>Acanthopagrus butcheri</i>	E	2.1	0.7	11.9	4.0	177	110-330	4.7	1.4	22.4	1.0	196	138-322
<i>Aldrichetta forsteri</i>	MEO	3.5	1.0	19.6	3.0	247	136-331	1.6	0.7	7.8	5.0	208	140-260
<i>Pelates octolineatus</i>	MEO	4.4	2.6	20.9	2.5	160	145-187	4.4	2.6	20.9	2.5	160	145-187
<i>Rhabdosargus sarba</i>	MEO	1.1	0.6	5.1	6.0	139	95-197	1.1	0.6	5.1	6.0	139	95-197
<i>Arripis georgianus</i>	MEO	0.3	0.2	1.2	7.0	191	182-203	0.3	0.2	1.2	7.0	191	182-203
<i>Sillago schomburgkii</i>	MEO	0.2	0.1	0.9	8.5	248	221-274	0.2	0.1	0.9	8.5	248	221-274
<i>Pomatomus saltatrix</i>	MEO	0.2	0.1	0.9	8.5	177	160-208	0.2	0.1	0.9	8.5	177	160-208
<i>Gerres subfasciatus</i>	MEO	0.1	0.1	0.6	10.5	171	164-177	0.1	0.1	0.6	10.5	171	164-177
<i>Sphyraena novaehollandiae</i>	MS	0.1	0.1	0.6	10.5	236	90-382	0.1	0.1	0.6	10.5	236	90-382
<i>Pseudorhombus jenynsii</i>	MEO	0.1	0.1	0.3	11.0	152	152-152	0.1	0.1	0.3	11.0	152	152-152
Number of species		4						12					
Total catch rate		17.8						20.9					
Total number of fish		285						335					
Number of samples		16						16					

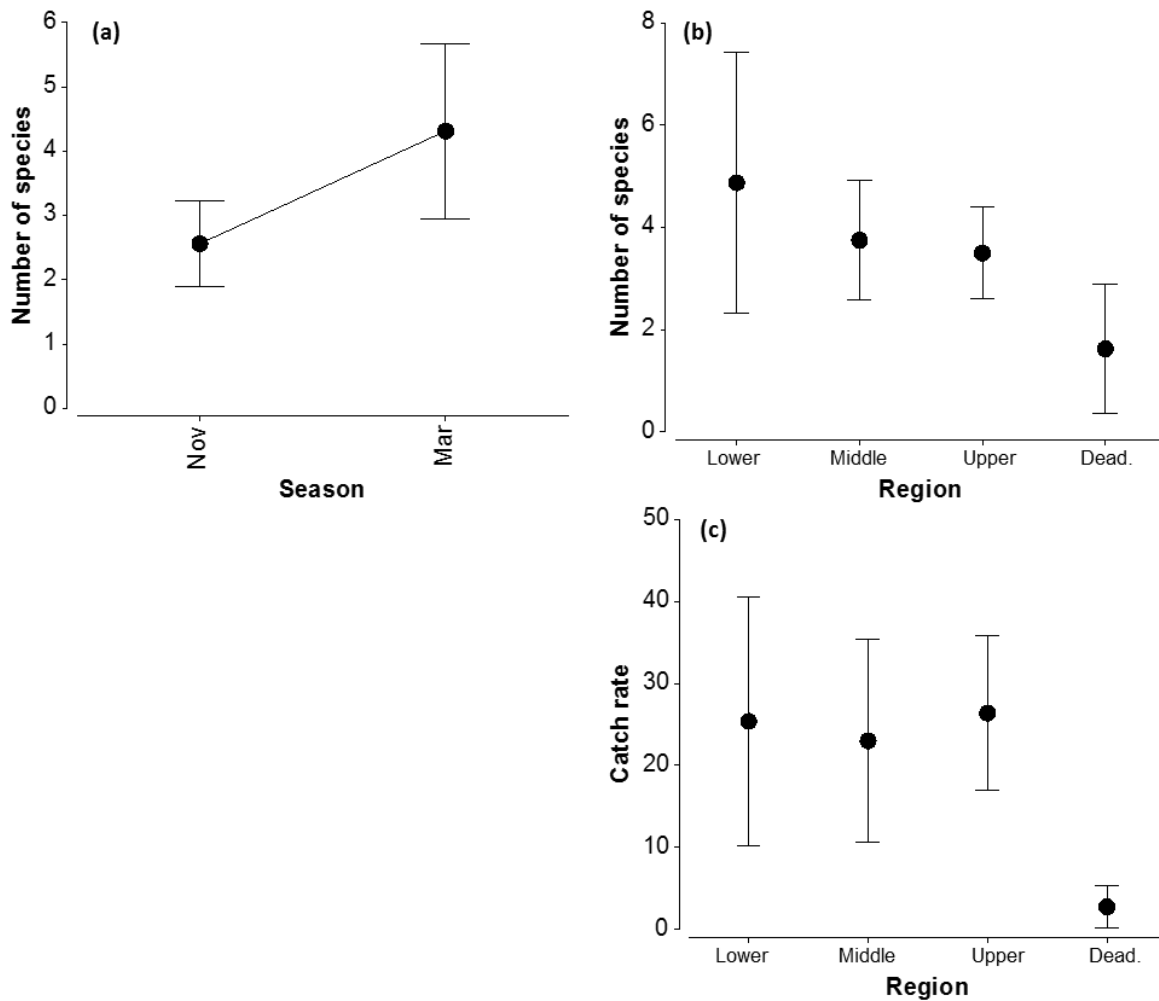


Fig. 8. Mean number of species in each (a) season and (b) region of Toby Inlet and mean (c) catch rate (h^{-1}) in each region. Error bars represent \pm 95% confidence limits.

Faunal composition

The composition of the fish fauna in the offshore waters of Toby Inlet was shown by PERMANOVA to differ significantly among both Season and Region, but not their interaction (Table 3c). The points representing both seasons form relatively widely-dispersed groups on the nMDS ordination, but those from each season mainly lie on opposite sides of the plot (Fig. 9a) While the intermingling of points from different seasons is likely due to the dominance of *M. cephalus*, *A. forsteri*, *A. caudavittata* and *A. butcheri* in the samples from both seasons, the broader spatial patterns reflect that fact that far more species were present in

samples collected in March, with *P. octolineatus* and *R. sarba* making substantial and, in the case of the latter species, also consistent, contributions to the fauna (Fig. 10a).

Fish faunal composition was shown to differ among all pairwise combination of regions except the lower vs middle estuary (Table 5). In particular, the upper estuary and Deadwater were the most distinct each forming a cluster of points on the left side of the nMDS ordination, with those for the lower estuary situated in the top right and above those of the middle estuary (Fig. 9b). The fish fauna of the Deadwater was the most distinct, due to its depauperate nature, containing only *A. caudavittata*, *A. butcheri*, *M. cephalus*, *A. forsteri*, and *R. sarba* (Fig. 10b). Moreover, the catches of the first two species were lower in the Deadwater than in the lower and middle estuary, likewise the two mugilid species were also less abundant in the Deadwater, particularly in comparison to the upper estuary. While the lower, middle and upper regions of Toby Inlet were all dominated by the same suite of species their abundances differed, with *A. caudavittata*, *A. butcheri* and *P. octolineatus* being caught in greater numbers in the lower and middle estuary, whereas the reverse was true for *M. cephalus* and *A. forsteri*.

Table 5. *t* statistic and significance level (*P*) values derived from a pairwise PERMANOVA test on the fish faunal composition of four regions of the offshore waters of Toby Inlet. Significant pairwise comparisons ($P < 0.05$) are highlighted in grey.

Region	Lower	Middle	Upper
Middle	0.904		
Upper	2.685	2.106	
Deadwater	2.015	2.012	2.235

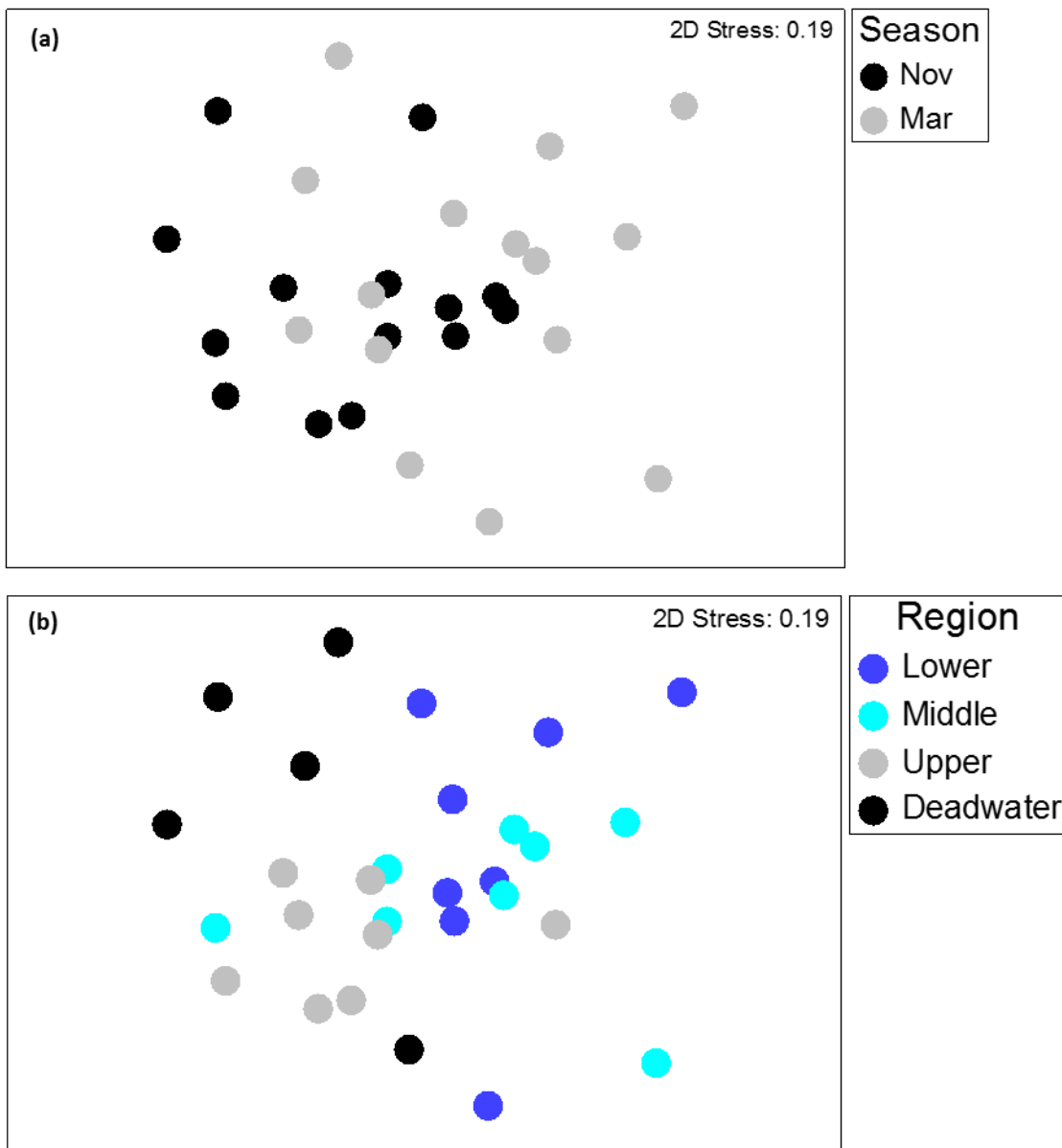


Fig. 9. Non-metric Multidimensional Scaling ordination plot, derived from a Bray-Curtis resemblance matrix of the dispersion-weighted and square-root transformed density of each fish species in the offshore waters of the four regions of Toby Inlet in November 2017 (bar closed) and March 2018 (bar open). Samples coded for (a) Season and (b) Region.

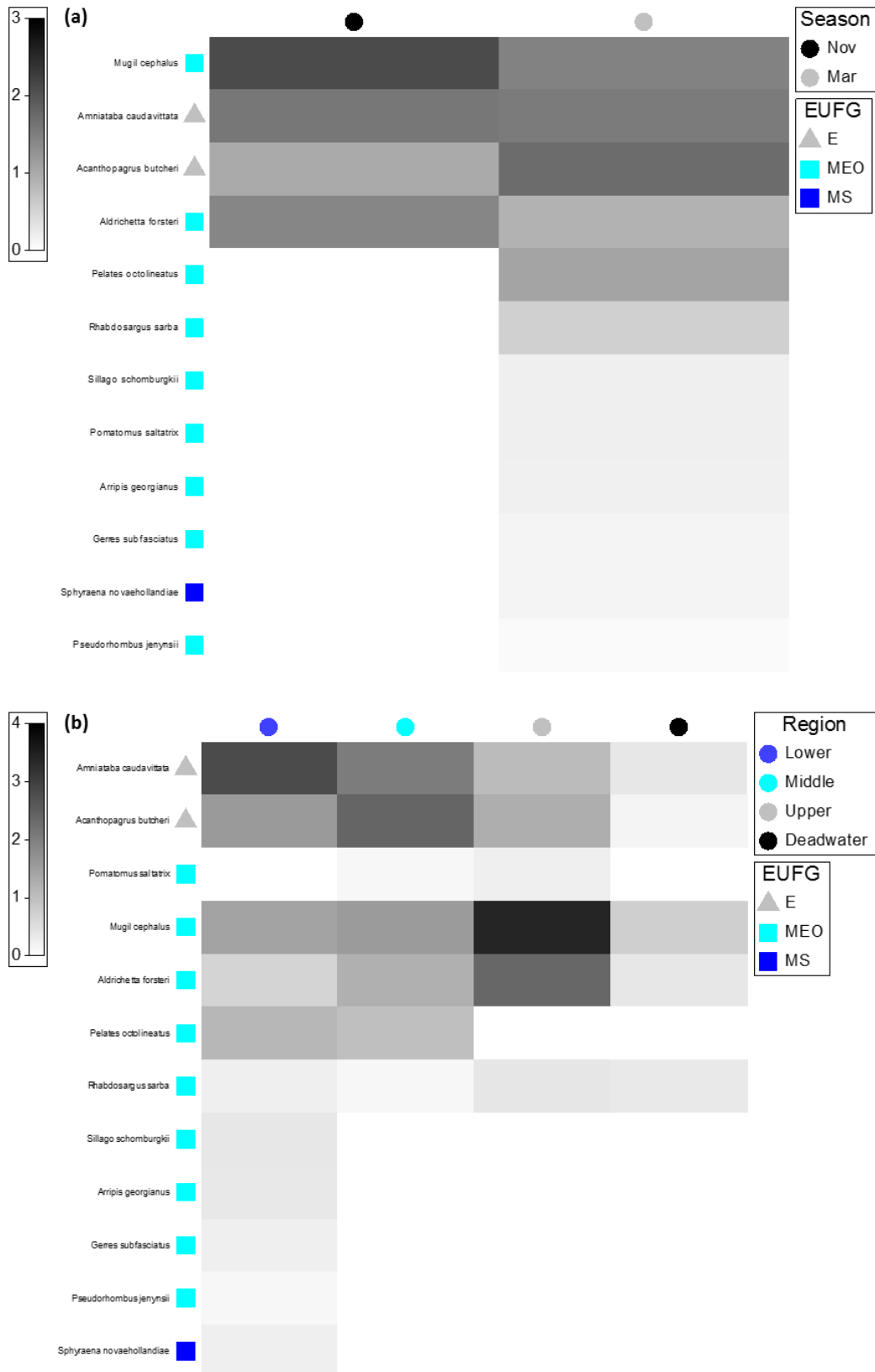


Fig. 10. Shade plot of the dispersion-weighted and square-root transformed average catch rates (h^{-1}) for each species recorded in the offshore waters of each (a) season and (b) region of Toby Inlet. White space indicates that a species was absent, and the shades of grey are proportional to the transformed abundances. The estuarine usage functional group (EUFG) to which each species belongs is also provided; see Table 3 for the full guilds.

Benthic macroinvertebrates

Contributions of invertebrate species

A total of 2,202 benthic macroinvertebrates were recorded from the 22 Ekman grab samples collected from the nearshore waters of Toby Inlet in November 2017 (Table 6). These invertebrates comprised 26 species from four subphylum. While the insects were the most speciose, constituting 12 species, members of this group contributed 23% to the total number of individuals, which is less than the five species of annelid and three species of mollusc that represented 32 and 28% of the total number of benthic invertebrates (Table 6). The top seven species all contributed > 5% to the total number of individuals and together contributing 90%. Among these the larvae of the non-biting midge *Chironominae* sp. 1 and the gastropod *Potamopyrgus* sp. were the most abundant species. Although not caught in the grab samples, other invertebrates recorded during the seine net sampling included the Southern Bobtail Squid *Euprymna tasmanica*, Blue Swimmer Crab *Portunus armatus* and Western King Prawn *Penaeus plebejus*.

Number of species, total density and diversity

The mean number of benthic macroinvertebrate species and their total density and Simpson's diversity did not differ significantly among regions ($P = 0.278-0.715$; Table 1d), largely due to considerable amounts of variability among the replicate samples. The mean values for the first of these biotic variables ranged from 6.5 in the Deadwater to 7.8 in the upper estuary up to 8.2 and 8.7 in the lower and middle estuary, respectively. While mean total density was less in the Deadwater compared to the three regions of Toby Inlet (*i.e.* 31 vs 44-52 individuals 100 cm⁻²), individual catches were highly variable, ranging from 0 and 1 to 104 and 107 individuals 100 cm⁻². Mean Simpson's diversity among regions ranged from 0.54 in the upper estuary, to 0.62 in the Deadwater to ~0.76 in the middle and lower estuary.

Table 6. Mean density (100 cm⁻²; X), standard error (SE), percentage contributions to the total catch (%C) and ranking based on abundance (R) of each benthic macroinvertebrate species caught in the nearshore waters of Toby Inlet in November 2017 (bar closed). Those species that contributed $\geq 5\%$ of the total catch are shaded in grey. The subphylum to which each species belongs is also provided.

Species	Subphylum	X	SE	%C	R
<i>Chironominae</i> sp. 1	Insecta	3.49	0.84	19.48	1
<i>Potamopyrgus</i> sp.	Mollusca	4.65	0.98	17.17	2
<i>Grandidierella propodentata</i>	Crustacea	1.88	0.64	13.99	3
<i>Arthritica semen</i>	Mollusca	4.24	1.45	10.72	4
<i>Simplisetia aequisetis</i>	Annelida	0.08	0.04	10.45	5
<i>Pseudopolydora kempfi</i>	Annelida	0.06	0.06	9.54	6
<i>Capitella capitata</i>	Annelida	0.02	0.02	7.86	7
<i>Scoloplos normalis</i>	Annelida	6.22	2.84	4.22	8
<i>Barnardomelita matilda</i>	Crustacea	0.89	0.33	2.00	9
<i>Chironominae</i> sp. 2	Insecta	0.04	0.03	1.41	10
Oniscidea sp.	Crustacea	0.30	0.16	0.68	11
<i>Chironominae</i> spp.	Insecta	0.10	0.06	0.50	12
Culicidae (pupa)	Insecta	8.67	3.89	0.50	12
<i>Coxiella striatula</i>	Mollusca	0.63	0.25	0.27	14
<i>Procladius</i> sp.	Insecta	0.22	0.11	0.23	15
<i>Ficopomatus enigmaticus</i>	Annelida	0.06	0.03	0.18	16
<i>Austrochiltonia subtenuis</i>	Crustacea	0.02	0.02	0.14	17
<i>Chironominae occidentalis</i>	Insecta	0.02	0.02	0.14	17
Odonta sp. 1	Insecta	0.22	0.09	0.14	17
Mysida sp.	Crustacea	0.02	0.02	0.09	20
Odonta spp.	Insecta	0.02	0.02	0.09	20
<i>Perthia</i> sp.	Crustacea	0.06	0.04	0.05	22
<i>Chironominae alternans</i>	Insecta	0.04	0.03	0.05	22
<i>Notalina spira</i>	Insecta	7.64	2.03	0.05	22
Aeshinidae sp.	Insecta	4.77	1.38	0.05	22
<i>Austrolestes annulosus</i>	Insecta	0.12	0.06	0.05	22
Number of species				26	
Total density				44.48	
Total number of invertebrates				2,202	
Number of samples				22	

Faunal composition

While no significant difference were detected in any of the univariate measures of diversity among regions, faunal composition did differ ($P = 0.001$; Table 1d). At a pairwise level, the four regions formed two groups, *i.e.* the lower and middle estuary (Table 7), located at the top of the nMDS ordination plot and the upper estuary and Deadwater, whose points are situated at the bottom of the plot. There were seven cosmopolitan species that were found in each

region, namely the annelids *Simplisetia aequisetis*, *Pseudopolydora kemp*i, *Capitella capitata* and *Scoloplos normalis*, the molluscs *Potamopyrgus* sp. and *Arthritica semen* and the insect larvae *Chironominae* sp. 2, albeit in varying densities. Differences between the two groups of regions were due to *A. semen*, *C. capitata*, *P. kemp*i and the amphipods *Barnardomelita matilda* being more abundant in the lower and middle estuary, whereas *Chironominae* sp. 1 was caught in consistent greater densities in the upper estuary as was the amphipod *Grandidierella propodentata* in the Deadwater.

Table 7. *t* statistic and significance level (*P*) values derived from a pairwise PERMANOVA test on the composition of the benthic macroinvertebrate fauna of four regions of the offshore waters of Toby Inlet. Significant pairwise comparisons (*P* < 0.05) are highlighted in grey.

Region	Lower	Middle	Upper
Middle	1.206		
Upper	2.148	1.938	
Deadwater	1.468	1.507	1.280

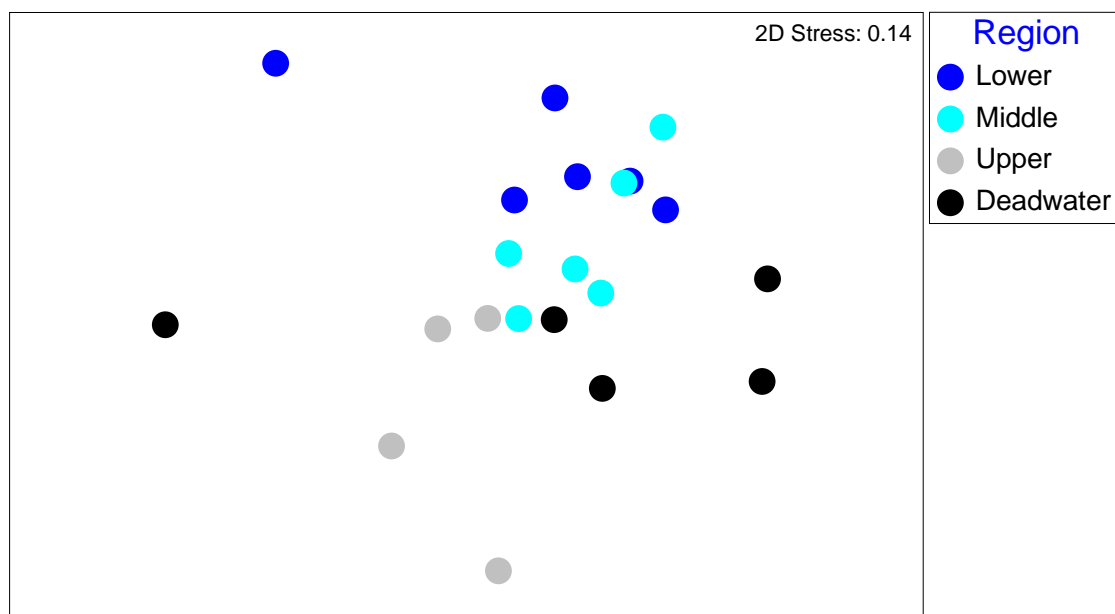


Fig. 11. Non-metric Multidimensional Scaling ordination plot, derived from a Bray-Curtis resemblance matrix of the dispersion-weighted and square-root transformed density of each benthic macroinvertebrate species in the nearshore waters of the four regions of Toby Inlet in November 2017.

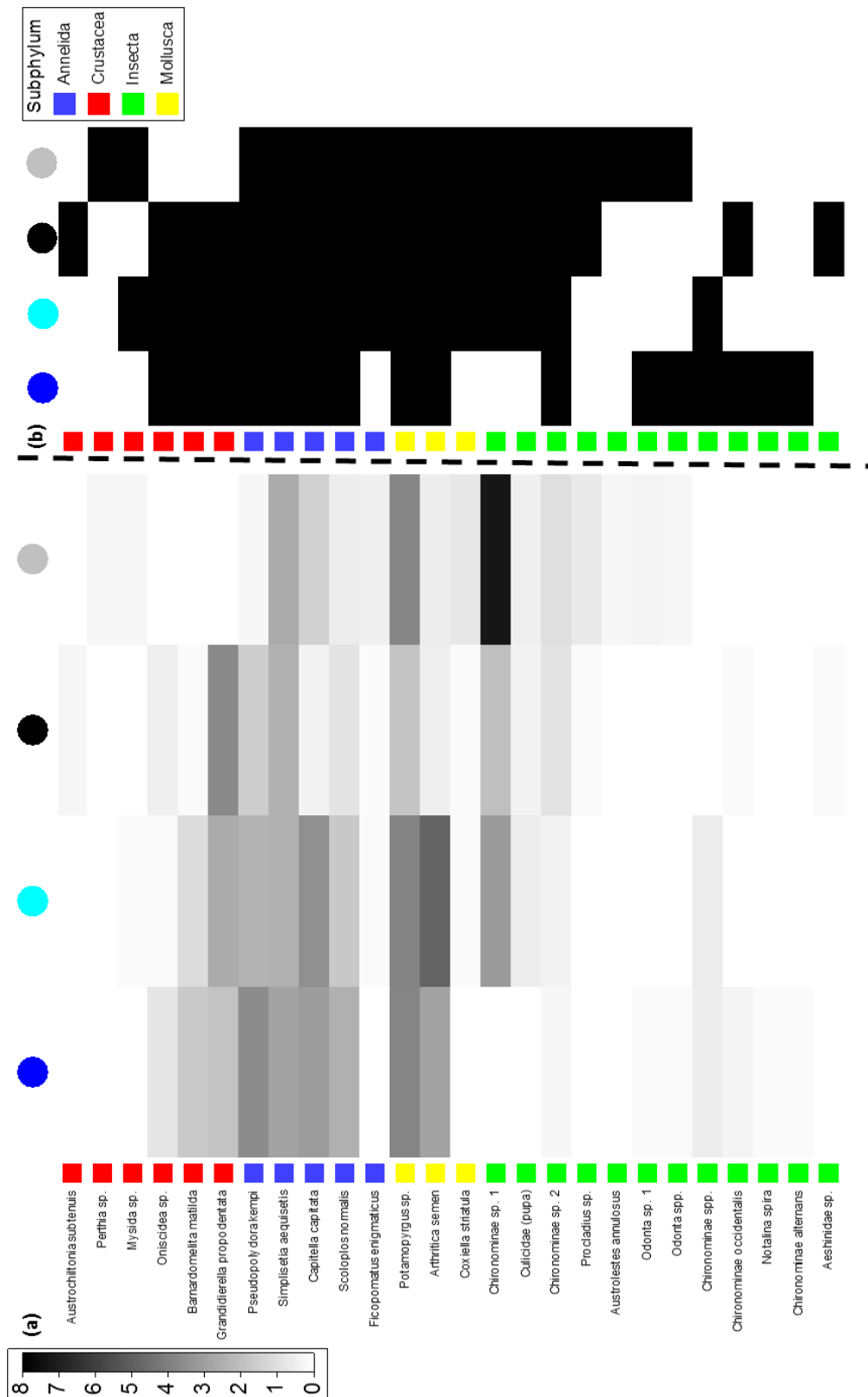


Fig. 12. (a) Shade plot of the dispersion-weighted and square-root transformed average densities (individuals 100 cm⁻²) for each benthic macroinvertebrate species recorded in the nearshore waters of Toby Inlet in November 2017 (bar closed). White space indicates that a species was absent, and the shades of grey are proportional to the transformed abundances. (b) The re-scaled version of (a) based on only presence (black) and absence (white) data. The subphylum to which each species belongs is also provided.

Discussion

This study aimed to determine, for the first time, the characteristics of the fish and benthic macroinvertebrate fauna of Toby Inlet, a small, very shallow, seasonally-open microtidal estuary in south-western Australia and whether they changed among regions. Focus was also placed on elucidating the impacts of artificially opening the bar at the mouth of Toby Inlet in summer on the fish fauna, as this management intervention has been suggested as a way of improving water quality during this time of year, when a lack of rainfall (and thus fluvial discharge), combined with the along-shore movement of sediment, results in a sand bar being formed across the mouth, disconnecting the estuary from the ocean (Frazer and Hall, 2018).

Water quality

On the basis of the salinity data provided by the Department of Water and Environmental Regulation, the salinity in the three regions of Toby Inlet in November 2017 (3-6) was less than in the previous two years, when it was 31-32 (2015) and 8-18 (2016). This trend was more pronounced in the Deadwater (1 during November 2017), but had not dropped below 24 previously during 'summer' months in 2015-2017. The salinity in either system (Toby Inlet or the Deadwater/Station Gully) reflects the timing and magnitude of fluvial discharge, which may or may not be sufficient to break the sand bar or produce enough scouring to maintain an existing breach (Brearley, 2005; Hoeksema et al., 2018).

In November 2017 the bars at the mouths of both parts of the system were intact, with that of Station Gully having formed relatively recently. In the case of the Deadwater, which had a salinity of 2, substantial fluvial discharge must have been entering from the Station Gully drain to lower salinity to such an extent. Salinities in the lower, middle and upper regions of Toby Inlet were also oligohaline, likely due to the build-up of freshwater from fluvial discharge, and increased in an upstream direction, thus forming a reverse salinity gradient. This pattern

runs counter to the typical paradigm, where salinity is greatest at the mouth of the estuary and decreases progressively upstream due to the declining influence of sea water penetration from tidal water movement and the increasing effect of freshwater input (Savenije, 2005; Potter et al., 2010). Reverse salinity gradients are uncommon globally, but do occur in both the nearby Leschenault and Vasse-Wonnerup estuaries, albeit due to different mechanisms (cf. Tweedley et al., 2014a; Veale et al., 2014). The likely cause of the slight reverse salinity gradient in Toby Inlet is the shallowness of the upper estuary, which may result in greater evaporation, and thus the concentration of salt (Fig. 2c), and the highly seasonally rainfall and thus fluvial discharge (Hodgkin and Hesp, 1998; Hallett et al., 2018).

While the reverse salinity gradient was relatively minor in November, *i.e.* a difference of ~ 2 (6-8), it became much more pronounced in March 2018, *i.e.* 15; 36-52. The opening of the bar in December, whilst aiding the flushing of the estuary increased salinity, this increase in salt concentration was further exacerbated by the lack of rainfall and discharge in these summer months and the high levels of evaporation. This resulted in salinities in the upper estuary becoming hypersaline. The artificial breaching of the bar at the mouth of Toby Inlet allows waters in the lower parts of the system to be exchanged with the ocean and so, while they too experience evaporation, the effects of salt concentration are mitigated by tidal exchange. It is thus relevant that the hydrological model estimated that, while water in the lower estuary would be exchanged daily, that residence time in the middle and upper estuaries would be considerably longer, *i.e.* 12-18 and 30 days, respectively (Fig. 13). That model also suggested that maintaining the bar of Toby Inlet open resulted in 72% of the system being flushed regularly as opposed to 36% if just the mouth at Station Gully was open (Frazer and Hall, 2018).



Fig. 13. Heat map showing the residence time (*i.e.* the time required for a hypothetical tracer introduced into the system to be reduced to a third of its original concentration) for waters in Toby Inlet and the Deadwater under a scenario where, as occurred in March 2018, the bar at the mouth of Toby Inlet was open, but that of Station Gully was closed, with the culvert between the two systems open. Values obtained from advection-dispersion module built for Toby Inlet using TUFLOW-FV by the Department of Water and Environmental Regulation. Taken from Frazer and Hall (2018).

Hypoxia is known to occur in microtidal south-western Australian estuaries (Kurup and Hamilton, 2002) and can influence the biology and ecology of fish (Watsham, 2016; Cottingham et al., 2018) and particularly benthic macroinvertebrates (Tweedley et al., 2014b; 2016a; 2018a). However, none of the dissolved oxygen concentrations recorded during the current study were $< 4 \text{ mg L}^{-1}$ and these values increased from a mean of 5.6 mg L^{-1} when the bar was closed to 8.5 mg L^{-1} when it was open. It should be noted that the values recorded in this study were obtained during the day, when oxygen concentrations are greatest, but that additional sampling done at night did not detect the presence of hypoxic conditions (Tweedley, unpublished data).

Nearshore fish fauna

The fish fauna of the nearshore waters of Toby Inlet comprised predominantly short-lived, small-bodied species (*i.e.* mean total length < 50 mm), including five atherinids and three gobiids, which together contributed 94% to the total number of fish recorded. The dominance of members of these two families is typical for the shallow waters of south-western Australian estuaries, in particularly those that become disconnected from the ocean for all or part of the year by a sand bar (Valesini et al., 2009; 2014; Potter et al., 2016). For example Hoeksema et al. (2009) found that atherinids and gobiids contributed between 92.9–99.7% of the total number of fish in five estuaries on the south coast of Western Australia. Nine of the 17 species recorded in Toby Inlet are able to complete their life-cycle in estuaries, with each of the six marine species recorded being known to use estuaries as nurse areas, thus highlighting the adaptation of these species to be able to utilise these environments and tolerate their changing physico-chemical conditions (Potter and Hyndes, 1999; Potter et al., 2015b).

One notable species recorded in the current study was the introduced freshwater poeciliid *G. holbrooki*, which was introduced to south-western Australia in 1934 and has been recorded in numerous rivers in the Geopraphe Bay region and the Vasse-Wonnerup Estuary ~20 km to the east (Morgan et al., 1998; Tweedley et al., 2017a). This species is known to behave in a highly antagonistic manner to other small fishes, causing damage to their fins and even death (Schoenherr, 1981; Rowe et al., 2007), and to predate on the eggs and larvae of fish and amphibians (Ivantsoff and Aarn, 1999; Pyke, 2005). Unlike the Vasse-Wonnerup, however, another introduced species, the Goldfish, *Carassius auratus* was not recorded (Beatty et al., 2017).

Neither the mean number of species, total density or Simpson's diversity differed among regions and/or seasons. This likely reflects the small size of the system at ~ 5.6 km in length,

the lack of pronounced difference in salinity among regions in November (2-8), the euryhaline nature of many of the estuarine species and the partitioning of species to various regions and presence of freshwater, estuarine and marine species. While the mean number of species was similar in both seasons, the total number of species increased by 36% when the bar was open. This reflects that fact that several marine estuarine-opportunist species such as *P. octolineatus*, *Sillago burrus*, *Pseudorhombus jenynsii* and *Atherinomorus vaigiensis* were only caught in this season and thus had taken advantage of being able to recruit to the estuary through the open bar, whereas the freshwater species *G. occidentalis* was only caught in November, likely due to this species being only able to tolerate mesohaline conditions (Beatty et al., 2011). The status of the bar has been shown to influence the fish fauna of microtidal estuaries on the south coast of South Africa (Vorwerk et al., 2003).

Although mean total density did not differ among seasons, the abundances of *L. wallacei*, *P. olorum* and *G. holbrooki* increased markedly in March. The first of these species spawns in October/November, with the 1+ fish undergoing mortality and the 0+ recruits being caught in December (Prince and Potter, 1983). This agrees with the current study where the mean total length of *L. wallacei* decreased from 40 mm in November to 33 in March. Both *P. olorum* and *G. holbrooki* spawn over the same spring/summer period (Pen and Potter, 1991b; Gill et al., 1996) and the increases in abundance were accompanied with a decrease in mean total length, indicating the present of numerous smaller individuals consequent with spawning and subsequent recruitment.

In November 2017, when the bars of both mouths were closed, the Deadwater contained a unique fish fauna, characterised by some species that can spawn in marine waters *i.e.* *R. sarba* and *F. lateralis* and that are usually found in the lower reaches of estuaries, but also several species that are able to breed in freshwater such as *G. occidentalis*, *P. olorum*, *A. suppositus*

(Pen and Potter, 1991a; Gill and Potter, 1993). The presence of these two suites of species is the result of the bar of Station Gully having closed relatively recently prior to sampling in November allowing, at that time, the recruitment of species from the ocean and the discharge of freshwater from Station Gully facilitating the movement of fish from the riverine environment. In contrast, the three regions of Toby Inlet, all contained a mixture of atherinids and gobiids, albeit there was some spatial segregation with *F. lateralis* and *P. olorum* identified as typifying species in the lower and upper estuaries, respectively, mirroring their spatial distribution in other estuaries (Potter and Hyndes, 1999; Tweedley et al., 2016b).

The presence of an open connection with the ocean in March allowed colonisation of the lower estuary by atherinids with marine affinities such as *Leptatherina presbyteroides* and *A. vaigiensis* and several marine estuarine opportunists e.g. *P. octolineatus* and *P. jenynsii*. The presence of these species declined in an upstream direction, with species such as *A. elongata* and, in particular, *P. olorum* and *L. wallacei* becoming more abundant. While this trend is present in estuaries with a traditional salinity gradient (Potter and Hyndes, 1999; Valesini et al., 2009), it is interesting that, at the time of sampling in March 2018, the upper estuary was hypersaline at a salinity of 52 and 15 greater than the lower estuary at around full strength seawater (36). The presence of these ‘traditional’ upper estuarine species in hypersaline waters of the upper reaches of Toby Inlet reflects the fact that they are highly euryhaline. For example, *P. olorum* and *L. wallacei* have been recorded in salinities of 60, 114 and that of *A. elongata* to 147 (Hoeksema et al., in prep.). Another species that characterised the hypersaline upper estuary was *G. holbrooki*, a freshwater species. While some studies report that this species intolerant of estuarine salinities (e.g. Alcaraz and García-Berthou, 2007; Alcaraz et al., 2008), others report that a congener, *Gambusia affinis*, is found in salinities up to 40 in the wild and has been acclimatised up to 59 in a laboratory setting and thus the presence of this species in waters at 52 may be one of the highest in vivo records (Chervinski, 1983; Hubbs, 2000).

Offshore fish fauna

The fish fauna of the offshore waters of Toby Inlet comprised predominantly marine estuarine-opportunist species, a characteristic of the fish fauna of south-western Australian estuaries (Chuwen et al., 2009a; Cottingham et al., 2015; Tweedley et al., 2016b). Two species that are able to complete their life cycle in estuaries were also recorded, with these representing ~40% of the total number of fish in both seasons. While the number of estuarine species stayed the same in both seasons (*i.e.* 2), the number of marine species increased from two in November, when the bar of Toby Inlet was closed, to ten in March, after the bar had been open for several months. This undoubtedly reflects the opportunity for such species to recruit to the estuary, which may be enhanced by the high salinities present in the lower reaches following the breaching of the bar in December and the lack of summer rainfall.

In addition to the total number of species being greater in March than November the same was true of the mean number of species, again likely a direct result of the bar having been opened for a period to allow recruitment of marine species into the estuary. For example, Chuwen et al. (2009a), found 27-29 species in the offshore waters of the basins of three seasonally-open estuaries compared to 17 in an estuary with more limited connectivity (*i.e.* normally-closed) and 45 in one with a permanently-open connection. A significantly greater number of species was recorded in the lower reaches of Toby Inlet and declined in an upstream direction, being lowest in the Deadwater. The upstream decline is due to many marine species only residing in the lower estuary, which is closest to the mouth where recruitment occurs and the region where salinities are most similar to seawater. Only a few of these species, *i.e.* the mugillids, penetrate the upper reaches of estuaries when they recruit (Chubb et al., 1981; Loneragan et al., 1987). The depauperate nature of the fauna in the Deadwater is the result of the bar at the mouth of that system remaining closed in both seasons, thus preventing direct recruitment from the

ocean, the provision of only a narrow channel and culvert connecting the Deadwater to the lower estuary (see title page) and the very shallow bathymetry of this region, which could make these larger, deeper-bodied species vulnerable to predation from the abundant bird life that resides on the estuary. These reasons also explain why the mean catch rate in the Deadwater was markedly lower than the three regions of Toby Inlet.

In terms of fauna composition, the seasonal differences observed were caused by increases in the number of marine estuarine-opportunists, but also changes in the abundances of several species. While both *P. octolineatus* and *R. sarba* were only recorded in March 2018, they were more abundant than many of the other marine species. It is thus relevant that during seasonal sampling of the Vasse-Wonnerup, where the mouth is typically open in winter (natural breach) and summer (artificial breach), the above species were both most abundant during summer (Cottingham et al., 2015). Moreover, in the permanently-open Peel-Harvey Estuary juvenile and older *P. octolineatus* were found to enter the estuary in summer (Veale et al., 2016). The abundance of not all species increased in March, however, with that of the two mugillid species, *i.e.* *M cephalus* and *A. forsteri*, decreasing. As these species are unable to reproduce in the estuary and their spawning seasons extend from March to September and from March to August, respectively, their reduced catch rate in March might be due to some individuals leaving the estuary to breed, compensated, to some extent, by the presence of some new recruits (Chubb et al., 1981).

Regional differences in species composition were due to the depauperate nature of Deadwater, which is caused by its lack of connectivity to the ocean (via the closed sand bar) and the small culvert limiting the connection to the lower region of Toby Inlet. The fauna of the upper estuary was relatively impoverished comprising predominantly mugilids, which are known to penetrate the upper reaches of estuaries and even the saline lower reaches of rivers (Chubb et al., 1981;

Chuwen et al., 2009a). In contrast the lower and, to a lesser extent, middle estuary contained greater numbers of marine estuarine-opportunists, although not always in larger abundances, reflecting the closeness of these regions to the ocean.

Benthic macroinvertebrates

The benthic macroinvertebrate fauna of estuaries typically comprises annelids, molluscs and crustaceans (Platell and Potter, 1996; Ysebaert et al., 2003; Tweedley et al., 2015) yet, while these subphyla were represented by 14 species in the samples from Toby Inlet, the remaining 12 species were the larval forms of insects. In addition to representing 42% of the species, they also contributed 23% to the total number of individuals. The presence of these species contrasts with the results obtained from the lower reaches of the Swan-Canning and Peel-Harvey estuaries (Wildsmith et al., 2009; 2011), but it similar to those from the upper reaches of the former system (Kanandjembo et al., 2001; Tweedley et al., 2016a) and of the nearby Vasse-Wonnerup (Tweedley et al., 2011; 2013). This likely reflects the low salinities present in Toby Inlet at the time of sampling and the close proximity of the system to the freshwater wetlands upstream of Cave Road Bridge.

Despite comprising only five and three species, 32 and 28% of the individuals belonged to the annelids and molluscs, respectively. The bivalve *A. semen* and polychaetes *S. aequisetis* and *P. kempi* each represented ~10% of the individuals and are common 'estuarine' species able to survive in the upstream reaches of the Swan-Canning Estuary (Kanandjembo et al., 2001; Tweedley et al., 2016a). In addition to the insects, several other 'wetland species were recorded including the gastropod *Potamopyrgus* sp. and the amphipods *Austrochiltonia subtenuis* and *Perthia* sp. (Davis and Christidis, 1997). While the fauna collected during November 2017 comprised a mixture of traditional estuarine species and those more commonly found in wetlands and the uppermost reaches of some estuaries, it is worth noting that the salinity in

November was oligohaline ranging between 2 and 8, and that the composition may be different in more saline conditions, like those recorded in March 2018.

While there was no significant difference the mean number of species, density and Simpson's diversity among regions, faunal composition did change with the lower and middle estuary containing a different assemblage to that in the upper estuary and Deadwater. Typically, a greater proportion of wetland species were found in the last two regions, e.g. *A. subtenuis* and *Perthia* sp. and the larvae of Chironominae sp. 1, whereas estuarine species, such as *A. semen*, *C. capitata*, *P. kempfi* and *B. matilda*, were more abundant in the lower reaches of the system mirroring their distribution in other systems in south-western Australia (Cheal et al., 1993; Davis and Christidis, 1997; Tweedley et al., 2014b). Given that the composition of the benthic macroinvertebrate fauna in estuaries has been shown to be influenced by salinity (Ysebaert et al., 1993; Basset et al., 2007; Dittmann et al., 2015), the results of the current study might differ markedly following a period of bar opening and/or the maintenance of higher salinities for a protracted period. It is thus relevant that identical sampling regimes conducted before and after the construction of a second and artificial entrance channel in the Peel-Harvey Estuary yielded 28 and 63 species of benthic macroinvertebrate, respectively (Wildsmith et al., 2009). The new species, which were typically marine, were thought to have been introduced as planktonic larvae from adjacent marine waters through the second channel and able to survive as salinity in the estuary had increased to close to that of the marine environment due to increase tidal exchange and flushing coupled with declining freshwater flow (Potter et al., 2016). Moreover, in microtidal estuaries in other parts of Australia and South Africa, the extent of connectivity between the ocean and the estuary has been shown to increase the number of species (Teske and Wooldridge, 2001; Dye and Barros, 2005).

Implications and future directions

This study determined, for the first time, the characteristics of the fish and invertebrate fauna of Toby Inlet (including the Deadwater). Fish living in the shallow, nearshore waters are predominantly short-lived, small-bodied estuarine species, which can complete their life-cycle in estuaries and are highly euryhaline. Thus, while opening of the bar allowed some species such as *L. presbyteroides* to colonise some sites in the lower reaches of Toby Inlet, the fauna as a whole changed little, being more influenced by the timing to reproduction of a number of key species. In contrast, the offshore fish fauna, which comprise mainly marine species, changed markedly following the opening of the bar, reflecting the opportunity to recruit to the estuary and the increases in salinity accompanied by greater tidal exchange. While the opening of the bar will typically result in greater numbers of larger fish from a wider range of species being present in the system and may help with flushing of nutrients, phytoplankton and potentially some sediment, it should be noted that artificially breaching of some intermittently-open estuaries in eastern Australia has been known to cause hypoxia and fish kills (Griffiths, 1999; Becker et al., 2009). Thus there is value in measuring water quality in the system, particularly the deeper waters on the upstream side of the bar prior to undertaking any breaching effort.

While the results of this study largely parallel the trends recorded in other estuaries in south-western Australia, it is worth noting that the sampling was conducted on only a single two-day occasion when the bar was closed and again when it was open and thus may not be reflective of all bar-state and season combinations, particularly immediately post breaching. This is emphasised by the fact that salinity was markedly lower in the spring and early summer of 2017 than in the previous two years. In the case of the benthic macroinvertebrate fauna, which was only sampled in November, this could have resulted in the presence of greater numbers of wetland taxa (*e.g.* insect larvae and some amphipods) having been flushed down into the

system and not suffering mortality due to the presence of low rather than high salinities. There would thus be value in repeating this survey at a time when salinities are higher to provide a truer reflection of the characteristics of the benthic macroinvertebrate fauna. Furthermore, as many infaunal species have a particular sediment preference, information on the sediment characteristics (*i.e.* grain size composition and organic matter content) would be useful in interpreting the results.

In the current study, all sampling was conducted downstream of Cave Road Bridge, however, the areas upstream are liable to be influenced if the recommendation made by Frazer and Hall (2018) to create a second artificial mouth in the upper estuary to reduce the residence time and prevalence of hypersalinity in that region is followed. This would potentially influence the water quality and fauna composition of the estuary and particular the upstream region for which we have no data to base predictions of the effects of this potential management option on.

This study generated the first data on the fish and invertebrate fauna of Toby Inlet. As these were collected at a relatively large number of sites given the size of the estuary, they provide a spatially robust benchmark against which future changes could be detected. This is particularly important given the development of the management plan for the system, which has the potential to impact on the system its water quality and flora and fauna.

References

- Alcaraz, C., García-Berthou, E., 2007. Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient. *Biological Conservation* 139, 83-92.
- Alcaraz, C., Bisazza, A., García-Berthou, E., 2008. Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* 155, 205-213.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK.
- Barbier, E.B., 2007. Valuing ecosystem services as productive inputs. *Economic Policy* 22, 178-229.
- Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2010. The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81, 169-193.
- Basset, A., Galuppo, N., Sabetta, L., 2007. Environmental heterogeneity and benthic macroinvertebrate guilds in Italian lagoons. *Transitional Waters Bulletin* 1, 48-63.
- Beatty, S.J., Morgan, D.L., Rashnavadi, M., Lymbery, A.J., 2011. Salinity tolerances of endemic freshwater fishes of south-western Australia: implications for conservation in a biodiversity hotspot. *Marine and Freshwater Research* 62, 91-100.
- Beatty, S.J., Allen, M.G., Whitty, J.M., Lymbery, A.J., Keleher, J.J., Tweedley, J.R., Ebner, B.C., Morgan, D.L., 2017. First evidence of spawning migration by goldfish (*Carassius auratus*); implications for control of a globally invasive species. *Ecology of Freshwater Fish* 26, 444-455.
- Beck, M.W., Heck Jr, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51, 633-641.
- Becker, A., Laurenson, L.J.B., Bishop, K., 2009. Artificial mouth opening fosters anoxic conditions that kill small estuarine fish. *Estuarine, Coastal and Shelf Science* 82, 566-572.
- Brearley, A., 2005. Ernest Hodgkin's Swanland, 1st ed. University of Western Australia Press, Crawley.
- Bricker, S.B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., Woerner, J., 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. *Harmful Algae* 8, 21-32.
- Cheal, F., Davis, J.A., Grouns, J.E., Bradley, J.S., Whittles, F.H., 1993. The influence of sampling method on the classification of wetland macroinvertebrate communities. *Hydrobiologia* 257, 47-56.
- Chervinski, J., 1983. Salinity tolerance of the mosquito fish, *Gambusia affinis* (Baird and Girard). *Journal of Fish Biology* 22, 9-11.
- Chubb, C.F., Potter, I.C., Grant, C.J., Lenanton, R.C.J., Wallace, J., 1981. Age, structure, growth rates and movements of sea mullet, *Mugil cephalus* L., and Yellow-eye Mullet, *Aldrichetta forsteri* (Valenciennes), in the Swan-Avon river system, Western Australia. *Marine and Freshwater Research* 32, 605-628.

- Chuwen, B.M., Hoeksema, S.D., Potter, I.C., 2009a. Factors influencing the characteristics of the fish faunas in offshore, deeper waters of permanently-open, seasonally-open and normally-closed estuaries. *Estuarine, Coastal and Shelf Science* 81, 279-295.
- Chuwen, B.M., Hoeksema, S.D., Potter, I.C., 2009b. The divergent environmental characteristics of permanently-open, seasonally-open and normally-closed estuaries of south-western Australia. *Estuarine, Coastal and Shelf Science* 85, 12-21.
- Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18, 117-143.
- Clarke, K.R., Chapman, M.G., Somerfield, P.J., Needham, H.R., 2006. Dispersion-based weighting of species counts in assemblage analyses. *Marine Ecology Progress Series* 320, 11-27.
- Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014a. *Change in Marine Communities: an Approach to Statistical Analysis and Interpretation*, 3 ed. PRIMER-E Ltd, Plymouth, UK.
- Clarke, K.R., Tweedley, J.R., Valesini, F.J., 2014b. Simple shade plots aid better long-term choices of data pre-treatment in multivariate assemblage studies. *Journal of the Marine Biological Association of the United Kingdom* 94, 1-16.
- Clarke, K.R., Gorley, R.N., 2015. *PRIMER v7: User Manual/Tutorial*. PRIMER-E, Plymouth.
- Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S., Turner, R.K., 2014. Changes in the global value of ecosystem services. *Global Environmental Change* 26, 152-158.
- Cottingham, A., Tweedley, J.R., Green, A.T., Green, T.A., Beatty, S.J., Potter, I.C., 2015. Key biological information for the management of Black Bream in the Vasse-Wonnerup. Murdoch University, Perth, Australia, p. 40.
- Cottingham, A., Hall, N.G., Hesp, S.A., Potter, I.C., 2018. Differential changes in production measures for an estuarine-resident sparid in deep and shallow waters following increases in hypoxia. *Estuarine, Coastal and Shelf Science* 202, 155-163.
- Creighton, C., Boon, P.I., Brookes, J.D., Sheaves, M., 2015. Repairing Australia's estuaries for improved fisheries production – what benefits, at what cost? *Marine and Freshwater Research* 66, 493-507.
- Davis, J., Christidis, F., 1997. *A guide to wetland invertebrates of southwestern Australia*. Western Australian Museum, Perth.
- Department of Water, 2010. *Vasse Wonnerup Wetlands and Geographe Bay water quality improvement plan*, p. 203.
- Dittmann, S., Baring, R., Baggalley, S., Cantin, A., Earl, J., Gannon, R., Keuning, J., Mayo, A., Navong, N., Nelson, M., Noble, W., Ramsdale, T., 2015. Drought and flood effects on macrobenthic communities in the estuary of Australia's largest river system. *Estuarine, Coastal and Shelf Science* 165, 36-51.
- Dye, A.H., Barros, F., 2005. Spatial patterns in meiobenthic assemblages in intermittently open/closed coastal lakes in New South Wales, Australia. *Estuarine, Coastal and Shelf Science* 62, 575-593.
- Elliott, M., Quintino, V., 2007. The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin* 54, 640-645.

- Fisher, B., Turner, R.K., Morling, P., 2009. Defining and classifying ecosystem services for decision making. *Ecological Economics* 68, 643-653.
- França, S., Costa, M.J., Cabral, H.N., 2009. Assessing habitat specific fish assemblages in estuaries along the Portuguese coast. *Estuarine, Coastal and Shelf Science* 83, 1-12.
- Frazer, J., Hall, J., 2018. Reconnecting Toby Inlet: options for increasing water circulation in Toby Inlet to improve water quality. Department of Water and Environmental Regulation, Perth, Western Australia., p. 58.
- Gill, H.S., Potter, I.C., 1993. Spatial segregation amongst goby species within an Australian estuary, with a comparison of the diets and salinity tolerance of the two most abundant species. *Marine Biology* 117, 515-526.
- Gill, H.S., Wise, B.S., Potter, I.C., Chaplin, J.A., 1996. Biannual spawning periods and resultant divergent patterns of growth in the estuarine goby *Pseudogobius olorum*: Temperature-induced? *Marine Biology* 125, 453-466.
- Griffiths, S.P., 1999. Consequences of artificially opening coastal lagoons on their fish assemblages. *International Journal of Salt Lake Research* 8, 307-327.
- Grixti, D., Morison, A., Bell, J.D., 2010. Undersized *Acanthopagrus butcheri* caught and released from commercial gill nets show high survival rates in the Gippsland Lakes, southeastern Australia. *North American Journal of Fisheries Management* 30, 723-731.
- Hallett, C.S., Hobday, A.J., Tweedley, J.R., Thompson, P.A., McMahan, K., Valesini, F.J., 2018. Observed and predicted impacts of climate change on the estuaries of south-western Australia, a Mediterranean climate region. *Regional Environmental Change* 18, 1357-1373.
- Hart, B.T., 2014. Independent review of the current and future management of water assests in the Geographe catchment, WA. Water Science, Echuca, Australia, p. 59.
- Hodgkin, E.P., Hesp, P., 1998. Estuaries to salt lakes: Holocene transformation of the estuarine ecosystems of south-western Australia. *Marine and Freshwater Research* 49, 183-201.
- Hoeksema, S.D., Chuwen, B.M., Potter, I.C., 2009. Comparisons between the characteristics of ichthyofaunas in nearshore waters of five estuaries with varying degrees of connectivity with the ocean. *Estuarine, Coastal and Shelf Science* 85, 22-35.
- Hoeksema, S.D., Chuwen, B.M., Tweedley, J.R., Potter, I.C., 2018. Factors influencing marked variations in the frequency and timing of bar breaching and salinity and oxygen regimes among normally-closed estuaries. *Estuarine, Coastal and Shelf Science* 208, 205-218.
- Hoeksema, S.D., Chuwen, B.M., Tweedley, J.R., Potter, I.C., in prep. Ichthyofaunal characteristics of normally-closed estuaries: relationship with opening of estuary mouth and extents of change in salinity and oxygen *Estuarine, Coastal and Shelf Science*.
- Hubbs, C., 2000. Survival of *Gambusia affinis* in a hostile environment. *Southwestern Naturalist* 45, 521-522.
- Ivantsoff, W., Aarn, 1999. Detection of predation on Australian native fishes by *Gambusia holbrooki*. *Marine and Freshwater Research* 50, 467-468.
- Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Jon, E., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293, 629-638.

- Kanandjembo, A.N., Platell, M.E., Potter, I.C., 2001. The benthic macroinvertebrate community of the upper reaches of an Australian estuary that undergoes marked seasonal changes in hydrology. *Hydrological Processes* 15, 2481-2501.
- Kennish, M.J., 2001. Coastal Salt Marsh Systems in the U.S.: A Review of Anthropogenic Impacts. *Journal of Coastal Research* 17, 731-748.
- Kennish, M.J., 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29, 78-107.
- Kurup, R.G., Hamilton, D.P., 2002. Flushing of dense, hypoxic water from a cavity of the Swan River Estuary, Western Australia. *Estuaries* 25, 908-915.
- Lek, E., Fairclough, D.V., Platell, M.E., Clarke, K.R., Tweedley, J.R., Potter, I.C., 2011. To what extent are the dietary compositions of three abundant, co-occurring labrid species different and related to latitude, habitat, body size and season? *Journal of Fish Biology* 78, 1913-1943.
- Loneragan, N.R., Potter, I.C., Lenanton, R.C.J., Caputi, N., 1987. Influence of environmental variables on the fish fauna of the deeper waters of a large Australian estuary. *Marine Biology* 94, 631-641.
- Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312, 1806-1809.
- Maes, J., Liqueste, C., Teller, A., Erhard, M., Paracchini, M.L., Barredo, J.I., Grizzetti, B., Cardoso, A., Somma, F., Petersen, J.-E., Meiner, A., Gelabert, E.R., Zal, N., Kristensen, P., Bastrup-Birk, A., Biala, K., Piroddi, C., Egoh, B., Degeorges, P., Fiorina, C., Santos-Martín, F., Naruševičius, V., Verboven, J., Pereira, H.M., Bengtsson, J., Gocheva, K., Marta-Pedroso, C., Snäll, T., Estreguil, C., San-Miguel-Ayanz, J., Pérez-Soba, M., Grêt-Regamey, A., Lillebø, A.I., Malak, D.A., Condé, S., Moen, J., Czucz, B., Drakou, E.G., Zulian, G., Lavalle, C., 2016. An indicator framework for assessing ecosystem services in support of the EU Biodiversity Strategy to 2020. *Ecosystem Services* 17, 14-23.
- Morgan, D.L., Gill, H.S., Potter, I.C., 1998. Distribution, identification and biology of freshwater fishes in south-western Australia. *Records of the Australian Museum Supplement* 56, 1-97.
- Pen, L.J., Potter, I.C., 1991a. Biology of the western minnow, *Galaxias occidentalis* Ogilby (Teleostei : Galaxiidae), in a south-western Australian river. *Hydrobiologia* 211, 77-88.
- Pen, L.J., Potter, I.C., 1991b. Reproduction, growth and diet of *Gambusia holbrooki* (Girard) in a temperate Australian river. *Aquatic Conservation: Marine and Freshwater Ecosystems* 1, 159-172.
- Platell, M.E., Potter, I.C., 1996. Influence of water depth, season, habitat and estuary location on the macrobenthic fauna of a seasonally closed estuary. *Journal of the Marine Biological Association of the United Kingdom* 76, 1-21.
- Potter, I.C., Hyndes, G.A., 1999. Characteristics of the ichthyofaunas of southwestern Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in temperate Australia: a review. *Austral Ecology* 24, 395-421.
- Potter, I.C., Chuwen, B.M., Hoeksema, S.D., Elliott, M., 2010. The concept of an estuary: a definition that incorporates systems which can become closed to the ocean and hypersaline. *Estuarine, Coastal and Shelf Science* 87, 497-500.

- Potter, I.C., Tweedley, J.R., Elliott, M., Whitfield, A.K., 2015a. The ways in which fish use estuaries: a refinement and expansion of the guild approach. *Fish and Fisheries* 16, 230-239.
- Potter, I.C., Warwick, R.M., Hall, N.G., Tweedley, J.R., 2015b. The physico-chemical characteristics, biota and fisheries of estuaries, in: Craig, J. (Ed.), *Freshwater Fisheries Ecology*. Wiley-Blackwell, pp. 48-79.
- Potter, I.C., Veale, L.J., Tweedley, J.R., Clarke, K.R., 2016. Decadal changes in the ichthyofauna of a eutrophic estuary following a remedial engineering modification and subsequent environmental shifts. *Estuarine, Coastal and Shelf Science* 181, 345-363.
- Prince, J., Potter, I., 1983. Life-cycle duration, growth and spawning times of five species of atherinidae (Teleostei) found in a Western Australian estuary. *Marine and Freshwater Research* 34, 287-301.
- Pyke, G., 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* 15, 339-365.
- Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences* 7, 585-619.
- Rowe, D.K., Smith, J.P., Baker, C., 2007. Agonistic interactions between *Gambusia affinis* and *Galaxias maculatus*: implications for whitebait fisheries in New Zealand rivers. *Journal of Applied Ichthyology* 23, 668-674.
- Savenije, H.H.G., 2005. *Salinity and Tides in Alluvial Estuaries*. Elsevier Science Ltd, Amsterdam.
- Schoenherr, A.A., 1981. The role of competition in the displacement of native fishes by introduced species, in: Naiman, R.J., Stolz, D.L. (Eds.), *Fishes in North American Deserts* Wiley Interscience, New York, pp. 173-203.
- Sheaves, M., Baker, R., Nagelkerken, I., Connolly, R.M., 2014. True value of estuarine and coastal nurseries for fish: incorporating complexity and dynamics. *Estuaries and Coasts*, 1-14.
- Somerfield, P.J., Clarke, K.R., Warwick, R.M., 2008. Simpson Index, in: Jørgensen, S.E., Fath, B.D. (Eds.), *Encyclopaedia of Ecology Vol 4*. Elsevier, Oxford, pp. 3252-3255.
- Teske, P.R., Wooldridge, T.H., 2001. A comparison of the macrobenthic faunas of permanently open and temporarily open/closed South African estuaries. *Hydrobiologia* 464, 227-243.
- Tweedley, J.R., Chambers, J.M., Potter, I.C., 2011. The benthic macroinvertebrate faunas of the Vasse-Wonnerup Estuary as indicators of environmental degradation. Report for the South West Catchments Council. Murdoch University, Perth, Western Australia, p. 41.
- Tweedley, J.R., Chambers, J.M., Paice, R., 2013. Sediment accumulation and resuspension in the Vasse-Wonnerup Wetlands and its relationship to internal nutrient cycling. Murdoch University, Perth, Australia, p. 54.
- Tweedley, J.R., Keleher, J., Cottingham, A., Beatty, S.J., Lymbery, A.J., 2014a. The fish fauna of the Vasse-Wonnerup and the impact of a substantial fish kill event. Murdoch University, Perth, Australia, p. 113.
- Tweedley, J.R., Warwick, R.M., Clarke, K.R., Potter, I.C., 2014b. Family-level AMBI is valid for use in the north-eastern Atlantic but not for assessing the health of microtidal Australian estuaries. *Estuarine, Coastal and Shelf Science* 141, 85-96.

- Tweedley, J.R., Warwick, R.M., Potter, I.C., 2015. Can biotic indicators distinguish between natural and anthropogenic environmental stress in estuaries? *Journal of Sea Research* 102, 10-21.
- Tweedley, J.R., Hallett, C.S., Warwick, R.M., Clarke, K.R., Potter, I.C., 2016a. The hypoxia that developed in a microtidal estuary following an extreme storm produced dramatic changes in the benthos. *Marine and Freshwater Research* 67, 327-341.
- Tweedley, J.R., Warwick, R.M., Potter, I.C., 2016b. The contrasting ecology of temperate macrotidal and microtidal estuaries. *Oceanography and Marine Biology: An Annual Review* 54, 73-171.
- Tweedley, J.R., Hallett, C.S., Beatty, S.J., 2017a. Baseline survey of the fish fauna of a highly eutrophic estuary and evidence for its colonisation by Goldfish (*Carassius auratus*). *International Aquatic Research*.
- Tweedley, J.R., Warwick, R.M., Hallett, C.S., Potter, I.C., 2017b. Fish-based indicators of estuarine condition that do not require reference data. *Estuarine, Coastal and Shelf Science* 191, 209-220.
- Tweedley, J.R., Cottingham, A., Beatty, S.J., 2018a. Benthic macroinvertebrate monitoring in the Vasse-Wonnerup Wetlands: March 2017. Report for the Department of Water and Environmental Regulation, Western Australia, Murdoch University, Perth, Western Australia, p. 47.
- Tweedley, J.R., Dittmann, S.R., Whitfield, A.K., Withers, K., Hoeksema, S.D., Potter, I.C., 2018b. Hypersalinity: global distribution, causes and effects on the biota of estuaries and lagoons, in: Wolanski, E., Day, J.H., Elliott, M., Ramachandran, R. (Eds.), *Coasts and Estuaries: The Future*. Elsevier, Amsterdam, The Netherlands, pp. XX-XX.
- Valesini, F.J., Coen, N.J., Wildsmith, M.D., Hourston, M., Tweedley, J.R., Hallett, C.S., Linke, T.E., Potter, I.C., 2009. Relationships between fish faunas and habitat type in south-western Australian estuaries. Project 2004/045. Final Report for Fisheries Research and Development Corporation. Murdoch University, Perth.
- Valesini, F.J., Tweedley, J.R., Clarke, K.R., Potter, I.C., 2014. The importance of regional, system-wide and local spatial scales in structuring temperate estuarine fish communities. *Estuaries and Coasts* 37, 525-547.
- Veale, L., Tweedley, J.R., Clarke, K.R., Hallett, C.S., Potter, I.C., 2014. Characteristics of the ichthyofauna of a temperate microtidal estuary with a reverse salinity gradient, including inter-decadal comparisons. *Journal of Fish Biology* 85, 1320-1354.
- Veale, L.J., Coulson, P.G., Hall, N.G., Potter, I.C., 2016. Biology of a marine estuarine-opportunist fish species in a microtidal estuary, including comparisons among decades and with coastal waters. *Marine and Freshwater Research* 67, 1128-1140.
- Vorwerk, P.D., Whitfield, A.K., Cowley, P.D., Paterson, A.P., 2003. The influence of selected environmental variables on fish assemblage structure in a range of southeast African estuaries. *Environmental Biology of Fishes* 66, 237-247.
- Ward, N.J., Bush, R.T., Burton, E.D., Appleyard, S., Wong, S., Sullivan, L.A., Cheeseman, P.J., 2010. Monosulfidic black ooze accumulations in sediments of the Geographe Bay area, Western Australia. *Marine Pollution Bulletin* 60, 2130-2136.

- Warwick, R.M., Tweedley, J.R., Potter, I.C., 2018. Microtidal estuaries warrant special management measures that recognise their critical vulnerability to pollution and climate change. *Marine Pollution Bulletin* 135, 41-46.
- Watsham, J., 2016. The influence of environmental factors on the habitat use of Black Bream *Acanthopagrus butcheri* and the implications of artificial oxygenation in the Swan-Canning Estuary. Murdoch University, Perth, Australia, p. 105.
- Wildsmith, M.D., Rose, T.H., Potter, I.C., Warwick, R.M., Clarke, K.R., Valesini, F.J., 2009. Changes in the benthic macroinvertebrate fauna of a large microtidal estuary following extreme modifications aimed at reducing eutrophication. *Marine Pollution Bulletin* 58, 1250-1262.
- Wildsmith, M.D., Rose, T.H., Potter, I.C., Warwick, R.M., Clarke, K.R., 2011. Benthic macroinvertebrates as indicators of environmental deterioration in a large microtidal estuary. *Marine Pollution Bulletin* 62, 525-538.
- Wilson, J.G., 1988. *The Biology of Estuarine Management*. Croom Helm, London.
- Ysebaert, T., Meire, P., Maes, D., Buijs, J., 1993. The benthic macrofauna along the estuarine gradient of the Schelde Estuary. *Netherlands Journal of Aquatic Ecology* 27, 327-341.
- Ysebaert, T., Herman, P.M.J., Meire, P., Craeymeersch, J., Verbeek, H., Heip, C.H.R., 2003. Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary, NW Europe. *Estuarine, Coastal and Shelf Science* 57, 335-355.